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THE BLACK-POINT DISEASE OF WHEAT

A. F. El-Helaly

(Accepted for publication June 10, 1947)

INTRODUCTION

In May, 1940, an outbreak of the black-point disease was first noticed by the writer in the fields of the Ministry of Agriculture at Sakha, Lower Egypt.

Certain preliminary observations were made on this disease. Samples of different varieties of wheat grown in different localities were then carefully examined.

All the material used in this investigation was taken from the experimental fields of the Cereal and the Propagation sections of the Ministry of Agriculture and of the Faculty of Agriculture, Farouk 1 University.

The work to be described in this paper was carried out during 1940–1943 in the laboratories of the Plant Pathology Section of the Ministry of Agriculture, Dokki, and of the Faculty of Agriculture of Farouk 1 University, Damanhour.

HISTORICAL REVIEW

Evans (4) recorded black-point disease of wheat in the Upper Mississippi Valley in 1921. She attributed the disease to a species of *Helminthosporium* resembling *H. sativum* P.K. and B. Curzi (2) found in association with the disease in Italy a species of *Alternaria* not hitherto recorded, and which was named *A. peglionii*. The infesting mycelium does not appear to be limited solely to the teguments of the scutellum, but extends across and under the pericarp and into the groove of the grain, frequently as far as the bearded apex. Rasella (9) mentioned that examination of wheat grains affected with "moucheture" from Morocco revealed in the discolored regions of the scutellum and the groove of the grain a fairly large, septate, and brown mycelium developing in the teguments but never penetrating deeper. He isolated species of *Alternaria* which he believed to be the same as *A. tenuis* and *A. peglionii*. Pasinetti (8) found that wheat of the awned Argentine variety San Martin was affected with a form of black point. He concluded that the discoloration of the scutellum was not due to an organism. The condition was regarded as purely physiological and resulting from adverse environmental (perhaps climatic) conditions. Ziling (11) stated that a disease similar to that known in Italy as "puntatura" and in Morocco as "moucheture" is known to have occurred in Siberia at least since 1914. Isolations from diseased grains germinated on filter paper yielded *Alternaria tenuis* in 82 to 95 per cent, *Helminthosporium sativum* in 15 to 60 per cent, species of *Fusarium* in about 4 per cent, and a number of unidentified fungi in some 2 per cent of the cases. Bacteria were not present. Laumont and Murat (5) stated that microscopical examination of clean and affected grains

belonging to a number of wheat varieties of different origins showed the almost constant presence in their external integuments of a mycelium which in culture usually produced a species of *Alternaria,* believed to be *A. peglionii. Helminthosporium sativum* has never been found on wheat in Algeria. Dastur (3) stated that a condition of wheat grains closely resembling that described as black point in the Argentine San Martin wheat by Pasinetti in 1931 occurred in the Central Provinces of India. The only important point of difference is that while no organisms were found by Pasinetti in the discolored grains, bacteria and species of *Helminthosporium, Cladosporium, Ophiobolus,* and *Fusarium* were shown to be present in the Indian wheats. In a few cases, however, black-point wheat grains failed to show the presence in them of any organism. Hyphae were observed inside the affected grains in great abundance in the funicle and in the pericarp in the central region of the grain furrow; they grow between the pericarp and the seed coat, where they form a kind of stroma, and also occur in the coleoptile and in crushed cells behind the scutellum. Machacek and Greaney (6) noticed that the disease was prevalent and of economic importance in Manitoba and that apparently *durum* wheats were more susceptible to it than *vulgare* varieties. It was further found that the fungi chiefly associated in Manitoba with this condition are *Alternaria tenuis, A. peglionii, Helminthosporium sativum,* and *H. teres.* They suggest the term "kernel smudge" for the condition. Connors (1) recorded that the disease, due chiefly to species of *Alternaria,* was more prevalent than usual in the Prairie Provinces of Canada. The condition caused the de-grading of 7.6 per cent of the ears in Manitoba, 2.4 per cent in Saskatchewan, and of 2 ears in 5000 in Alberta.

SYMPTOMS OF THE DISEASE

The black-point or kernel-smudge disease of wheat is characterized by a brown diffuse discoloration of varying intensity, limited to the embryo region in the grain. It, often, has a narrow brown line extending along the groove, in some cases to the brush end of the kernel.

CAUSE OF THE DISEASE

Numerous isolations from superficially disinfected diseased grains in Egypt have given a species of *Alternaria.*

On examining sections made at the discolored parts of the grain, mycelium was seen in a few cases. It was located in the pericarp and penetrated to the integument. No invading hyphae have been seen in the aleurone layer, the starch cells of the endosperm, or the embryo.

The same fungus was also isolated from a few superficially sterilized kernels that were apparently healthy.

OCCURRENCE AND DISTRIBUTION OF THE DISEASE

The black-point disease has probably been present in Egypt for a long time, though there is no mention of the disease in the Egyptian literature.

Examination of samples of different varieties of wheat grain grown in different localities all over Egypt during 1940–1943 showed that the disease, in some of the varieties tested, is prevalent in the northern part of Lower Egypt and that the intensity of the disease decreases gradually southwards becoming rare in Upper Egypt.

In Giza 114, one of the very susceptible varieties, we find that the percentage of the disease in the crop of May, 1940, amounted to about 40 in the northern part of Lower Egypt, 30 in the southern part, 12 in Middle Egypt, and 1.5 in Upper Egypt.

The intensity of the disease varies from year to year and with the different varieties of wheat.

When the disease is severe and prevalent it becomes of some economic importance because it lowers greatly the grade of the grain. The disease has no harmful effect on the germination of the affected seeds, the emergence of the seedlings, or the yield of the subsequent crop.

FACTORS INFLUENCING DISTRIBUTION OF THE DISEASE

The spread and distribution of the disease in Egypt depend on a number of factors which act either together or separately. Some of these factors are external while the others are internal.

External Factors

Presence of the Spores of the Casual Organism in the Air. Infection, in this disease, results from air-borne spores which come in contact with the exposed part of the grain when conditions are favorable. This was ascertained by keeping certain immature ears of some susceptible plants under controlled conditions to prevent infection from the air as much as possible. When these were ripe the grains were apparently clean while the rest of the ears which were left to ripen naturally while exposed to the air contained some discolored grains.

Machacek and Greaney (6) working on this disease stated that the cereal grains, under Manitoba conditions, are infected by air-borne spores of the fungi. It was demonstrated by means of spore-traps that the largest numbers of such spores usually occur in the air about the time the grains are maturing.

Atmospheric Humidity and Rainfall. Atmospheric humidity and rainfall seem to favor the occurrence of the disease. There is comparatively more rain in the northern part of Lower Egypt than in the southern part, while there is very little rain in Middle Egypt, and practically no rain in Upper Egypt. The intensity of the disease in Egypt follows that of the rainfall. Table 1 contains the percentage of the disease in different localities as well as the amount of rainfall and the relative humidity in each of these localities.

Soil Humidity. There is no doubt that the amount of water given to the soil during the growing period of the crop influences the incidence of the

disease. It was observed by Pantanelli (7) that infection was very severe in cases where much water was absorbed by the plant, in proportion to the weight of its absorptive system, during the period immediately preceding inoculation. He also states that an increased concentration of the nutrient solution diminishes susceptibility in so far as it reduces the absorptive activity of the roots.

In Assiut Province in Egypt two systems of irrigation are applied. One area of this province is under the perennial system of irrigation: the crop is given more than one watering during its growing season. The other area is

TABLE 1.—*The percentage of black point in two varieties of wheat and relative humidity and monthly rainfall at different localities in Egypt in 1941*

Locality	Percentage black-point infection		Mean daily relative humidity (per cent)			Monthly rainfall (mm.)		
	Giza 114	Baladi 116	Mar.	Apr.	May	Mar.	Apr.	May
Sakha	62.5	20.0	84	72	62	22.2	Tr.	0.0
Damanhour	50.0	18.0	77	69	57	23.1	0.1	0.0
Mansura	50.0	13.0	80	69	57	27.0	Tr.	0.0
Gemmeza	31.5	12.0	88	76	71	19.2	Tr.	0.0
Giza	16.5	8.0	68	59	48	9.5	Tr.	Tr.
Minya	11.0	6.0	52	43	36	Tr.	Tr.	0.0
Assiut	1.5	1.5	51	39	30	0.0	0.0	0.0
Quena	1.5	1.0	42	32	24	0.0	Tr.	Tr.
Aswan	2.0	1.0	21	16	11	0.0	3.0	Tr.

basin lands and is under the flooding system of irrigation: the crop receives only one watering at the time of sowing. In the first area the incidence of the disease is rather higher and more noticeable than in the latter area where the disease is very light and even rare.

The data in table 2 were taken from two experiments, one in Mallawi which falls within the first area and the other in Rifa, a village, close to Assiut, which lies within the second area.

TABLE 2.—*The percentage of black point in four varieties of wheat grown at Mallawi where there were several irrigations during the season and at Rifa where a single flooding irrigated the crop*

Variety	Percentage of infection at	
	Mallawi	Rifa
Giza 114	12.5	1.5
Baladi 116	7.5	1.5
Giza 121 (Mabrouk)	4.5	1.5
Hindi 62	3.5	1.5

Soil humidity favors the disease attack. It also appears from the results in table 2 that the intensity of the disease varies with the different varieties of wheat.

Internal Factors

Size of Wheat Grain. It has been observed during this investigation that the size of wheat grain plays an important part in the incidence of the disease. Larger grains show usually heavier attack. Ziling (11) mentioned that wheat grains infected with *Alternaria tenuis* were readily distinguishable from those infected with *Helminthosporium sativum* in that the former were usually larger and heavier, while the latter were smaller and lighter than usual. Waldron (10) recorded that the weight of the black-point kernels on any one plant was generally greater than that of the apparently non-infected kernels. These results are considered to indicate that a portion of the weight differences is caused by a stimulation of the development of the endosperm, following the penetration of the fungus into the developing ovule, although they may also be attributed in part to a difference in infection of grains differing normally in size because of their position in the ear. Machacek and Greaney (6) stated that the largest grains in the ear are frequently infected while the small, shrunken ones are usually free from the disease.

VARIETAL RESISTANCE UNDER NATURAL INFECTION

Some varieties appear to be more susceptible to black-point disease than others (Table 2). A more extensive test on a number of varieties was carried out.

Grain samples of different varieties of wheat were taken from the crops resulting from many variety experiments made all over Egypt. Examination of these samples showed that some of the varieties tested were more affected than the others. Data on grain samples from two such experiments, at Sakha and at Gemmeza in Lower Egypt where the disease is prevalent, appear in table 3.

TABLE 3.—*The percentage of black point in grain samples of several varieties of wheat grown at two locations in Lower Egypt where the disease is prevalent*

Variety	Percentage of infected grains in samples from	
	Sakha[a]	Gemmeza[a]
Giza 114	62.5	31.5
Giza 115	60	20
Giza 116	44	20
Giza 74	48	12
Baladi 116	20	12
Giza 121 (Mabrouk)	12	8
Giza 100	13	3
Hindi 62	12	2
Giza 102	10	3
Giza 7	10	3
Hindi D	5	2

[a] Sakha is in the northern part of Lower Egypt, Gemmeza is in the southern part.

Hence, among the 11 varieties examined the least susceptible to black-point disease is Hindi D followed by Giza 7, Giza 102, and Giza 121 (Ma-

brouk). The varieties Giza 114, Giza 115, Giza 116, and Giza 74 are the most susceptible. Baladi 116 is partially susceptible.

MECHANISM OF RESISTANCE

The statement of Machacek and Greaney (6) may explain the mechanism of resistance to black-point disease in wheat. They stated that the largest grains in the ear are frequently infected while the small, shrunken ones are usually free from infection. This apparently is due to the fact that the large kernels force open their covering glumes, thus affording access to the spores, whereas the glumes of the small kernels remain closed and exclude the spores.

The volume of a known number of grains of certain resistant and susceptible wheat varieties was measured, and the percentage of the disease was estimated in each variety. The data obtained are in table 4.

TABLE 4.—*The volume of 1000 kernels and the percentage of black point in seven varieties of wheat*

Variety	Av. volume (cc.) of 1000 kernels	Percentage of infection
Giza 114	39	60
Giza 116	39	45
Giza 74	37	45
Baladi 116	30	22
Giza 121 (Mabrouk)	27	13
Hindi 62	28	13
Hindi D	25	5

The results illustrate that the most susceptible varieties, Giza 114, Giza 116, and Giza 74, have large kernels. Kernel volume is least in the most resistant variety, Hindi D; while the moderately susceptible variety Baladi 116 has an intermediate kernel volume.

Diseased and apparently healthy grains found in samples of certain varieties of wheat were separated from each other. The volume of each lot in each variety was determined. The results are recorded in table 5. The diseased grains were invariably larger than the apparently healthy ones.

TABLE 5.—*The volume of 1000 healthy kernels and of 1000 black-point kernels in grain samples of wheat varieties grown at Giza in 1941–42 and at Damanhour in 1942–43*

Variety and source of sample	Black point (per cent)	Av. volume (cc.) of 1000 kernels	
		Healthy	Infected
Baladi 116			
Giza, 1941–42	7.5	30.0	40.0
Damanhour, 1942–43	21.0	29.5	38.5
Hindi 62			
Giza, 1941–42	4.5	25.0	35.0
Giza 121			
Giza, 1941–42	1.5	25.0	35.5
Damanhour, 1942–43	1.0	27.5	40.0

It is, therefore, clear that the size of the grain is directly associated with the incidence of the disease. Resistance of some varieties of wheat to black-point disease is a morphological condition depending on the grains and their covering glumes. Resistant varieties produce small grains which have no effect on the glumes. The glumes, therefore, remain closed and do not permit the passage of the spores to the grain. Thus, infection is avoided even when all external conditions favor it.

It has been noticed, in some cases, that percentage of infection may vary in the same variety even when grown in the same locality. Investigation has shown that rust disease is the limiting factor in this case. It affects the grains which become small and shrunken and thus avoid infection by black-point disease so that the variety gains resistance.

SUMMARY

Black-point disease of wheat in Egypt was found to be due to species of *Alternaria*.

The disease is widely distributed in Lower Egypt. It decreases gradually southwards becoming rare in Upper Egypt.

The disease has no harmful effect on the germination of the affected grain. It only lowers the grade.

The spread and distribution of the disease in Egypt depend on a number of factors. The most important of these factors are presence of the spores of the casual organism in the air, atmospheric humidity and rainfall, soil humidity, and size of the grain.

Of the varieties of wheat tested with regard to differential susceptibility, Hindi D proved to be the least susceptible followed by Giza 7, Giza 102, and Giza 121 (Mabrouk). Giza 114, Giza 115, Giza 116, and Giza 74 were the most susceptible. Baladi 116 is partially susceptible.

The size of the grain affects directly the incidence of the disease. The large kernels force open their covering glumes, thus affording access to the spores, whereas the glumes of small kernels remain closed and, thus, avoid infection even when all conditions favor it.

The resistance of some varieties of wheat is, mainly, due to the fact that they produce small grains.

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FACULTY OF AGRICULTURE,
FAROUK 1 UNIVERSITY,
ALEXANDRIA, EGYPT.

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A STRAIN OF THE ALFALFA MOSAIC VIRUS ON PEPPER IN ONTARIO[1]

G. H. BERKELEY[2]

(Accepted for publication June 16, 1947)

In Brant and Lincoln counties, in 1943 and again in 1944, an unusual mosaic disease of pepper (California Wonder variety) was observed. In addition to mottling, chlorotic rings, spots, and patterns appeared on both leaves and fruit. These symptoms suggested that a virus was involved other than those found commonly on pepper in Ontario, namely, tobacco mosaic and cucumber mosaic. Preliminary inoculations indicated that a strain of *Marmor medicaginis* H. var. *typicum* was responsible for the unusual symptoms. So far as the author is aware, this is the first occurrence of this disease appearing naturally on sweet peppers.

EARLIER INVESTIGATIONS

Porter (5, 6) and Dykstra (2) have reported pepper as a host of the potato-calico virus, which Black and Price (1) consider to be *Marmor medicaginis* var. *solani.* Though Zaumeyer (8) and Zaumeyer and Wade (9, 10), have made comprehensive studies of the alfalfa-mosaic viruses, they did not include pepper in their range of hosts. In 1942, *M. medicaginis* was reported by Kovacevski (3) as occurring on Chili peppers in Bulgaria and by Snyder and Rich (7) on celery in California.

MATERIAL AND METHODS

Several isolates of pepper virus were obtained from naturally affected peppers in both Brant and Lincoln counties. As those from all sources produced similar symptoms on hosts within a restricted range, one was chosen for use throughout this investigation. For comparative purposes *Marmor medicaginis* H. var. *typicum* and *M. medicaginis* var. *solani,* the potato-calico strain, were kindly supplied by Dr. F. O. Holmes.

The differential host range included seedlings of *Nicotiana tabacum* L., var. Harrow Velvet; *N. glutinosa* L.; *N. rustica* L.; *Vicia faba* L., broad bean, var. Windsor Long Pod; *Phaseolus vulgaris* L., bean, var. Stringless Evergreen; *Lathyrus odoratus* L., sweet pea, var. Pinkie; *Pisum sativum* L., pea, var. Laxton's Progress or Dwarf Telephone; *Trifolium pratense* L., red clover; *Capsicum frutescens* L., pepper, var. California Wonder; *Soya max* (L.) Piper, soybean, var. Blackeye; *Lycopersicon esculentum* Mill., tomato, var. Grand Rapids; *Datura stramonium* L.; *Antirrhinum majus* L., snapdragon, var. Empress; *Solanum Melongena* L., eggplant, var. Black Beauty; *Cucumis sativus* L., cucumber, var. Windermoor Wonder; *Petunia*

[1] Contribution No. 903 from the Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada.

[2] Officer-in-charge, Dominion Laboratory of Plant Pathology, St. Catharines, Ontario.

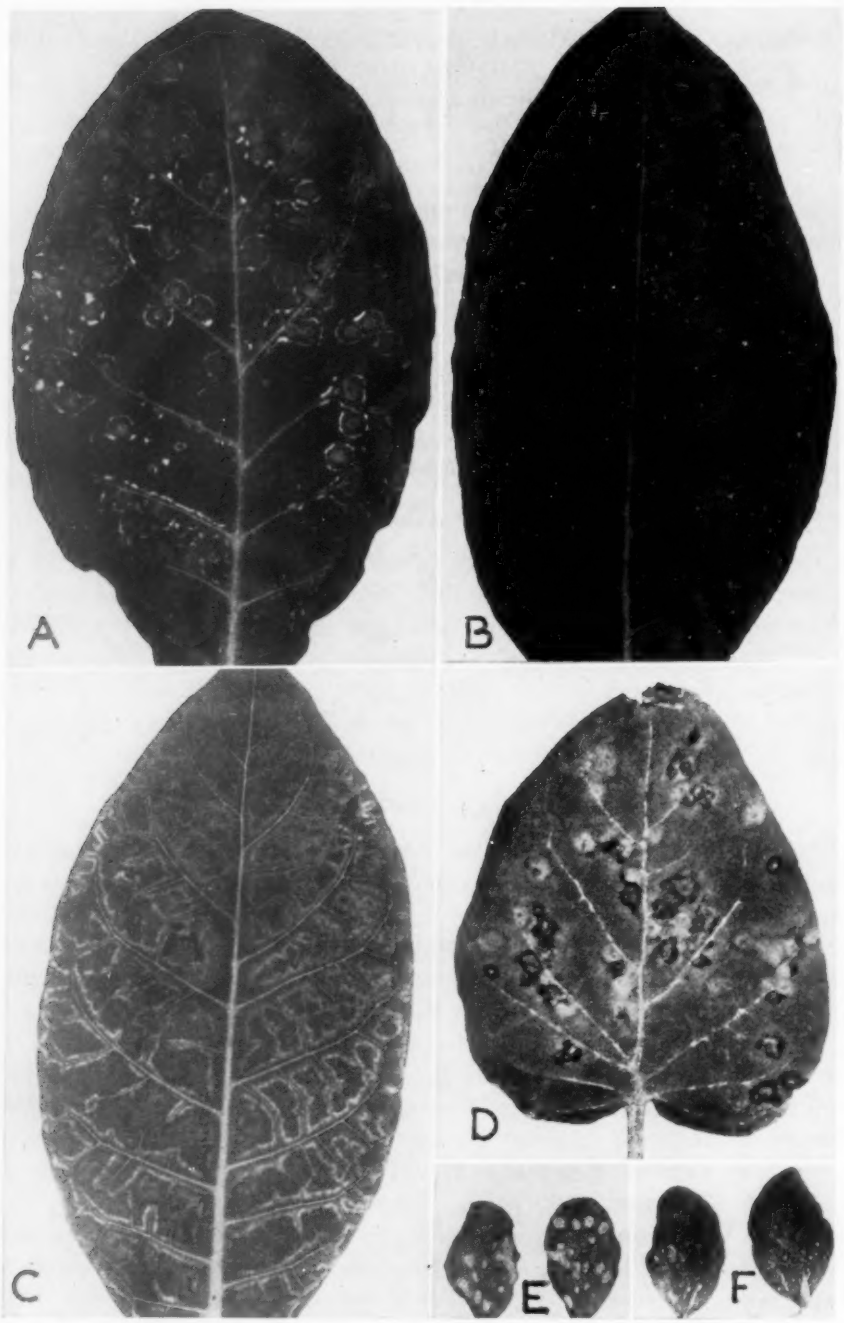

FIG. 1. Symptoms of pepper virus on various hosts. A: necrotic spots and rings on *Nicotiana tabacum;* B: systemic flecking on *N. tabacum;* C: vein-banding on *N. tabacum;* D: chlorotic and necrotic spots and rings on *N. glutinosa;* E: necrotic local lesions on sweet pea; F: systemic necrosis on sweet pea.

hybrida Viln., petunia; *Zinnia elegans* Jacq., zinnia; and *Apium graveolens* L., celery, var. Golden Plume.

Rapidly growing seedlings of a 4- to 6-leaf stage were generally used for inoculation which was effected by the cotton swab-carborundum technique, using unfiltered juice obtained by crushing fresh leaf material in a mortar and pestle.

Inoculations extended over a 3-year period, so that a wide range of environmental conditions was encountered.

SYMPTOMS PRODUCED BY THE PEPPER VIRUS

Before discussing the symptoms in detail for each host, it should be pointed out that there was considerable variation in this regard. For instance, only primary yellow lesions developed on the inoculated leaves in 2 series though in 14 series necrotic spots and rings were also formed. In some series both necrotic spots and rings developed, whereas in others only chlorotic or necrotic rings resulted. The most important single factor in this connection appeared to be the age of the rubbed leaves. For instance, in several series where the lowest leaf and two leaves above in order were inoculated on the same plant (*Nicotiana tabacum*), the symptoms on the bottom leaf were of the solid necrotic lesion type while, on the two upper leaves, primary yellow lesions with grayish-white necrotic rings developing around the primary yellow lesion were general, with solid necrotic lesions usually absent. The same effect was obtained on *N. rustica* when the lower leaf showed large, black, solid necrotic lesions while on the upper leaves smaller white papery necrotic lesions were formed.

Nicotiana tabacum. Sixteen series of inoculations involving 60 plants were carried out on this host. The general symptoms were primary yellow lesions with or without necrotic centers and grayish white necrotic rings followed by systemic vein-clearing, vein-banding, chlorotic mottling, and white necrotic flecks, rings and/or patterns (Fig. 1, A, B, and C). On plants held for several weeks a chlorotic mottling with some necrotic flecking was the predominant symptom on the later formed leaves.

When old slow-growing plants were inoculated, the symptoms were often very mild, with yellow lesions as the primary symptom and little or no necrotic flecking.

Nicotiana glutinosa. Fourteen series of inoculations, involving 52 plants, were effected on *N. glutinosa.* The resulting symptoms consisted of systemic vein-clearing, vein-banding, and a green-spot type of mottle with necrotic flecks and lines. On the rubbed leaves the symptoms varied considerably. Sometimes symptoms were lacking while at other times yellow lesions, which generally became necrotic, developed before, at about the same time, or after the first systemic symptoms (Fig. 1, D). On plants held for several weeks, chlorotic mottling with mild necrosis became the predominant symptom on later formed leaves.

Nicotiana rustica. On this host fifteen series of inoculations, involving 55 plants, were effected. Symptoms consisted of primary yellow lesions, grayish-white etched rings, and/or concentric rings, followed by systemic

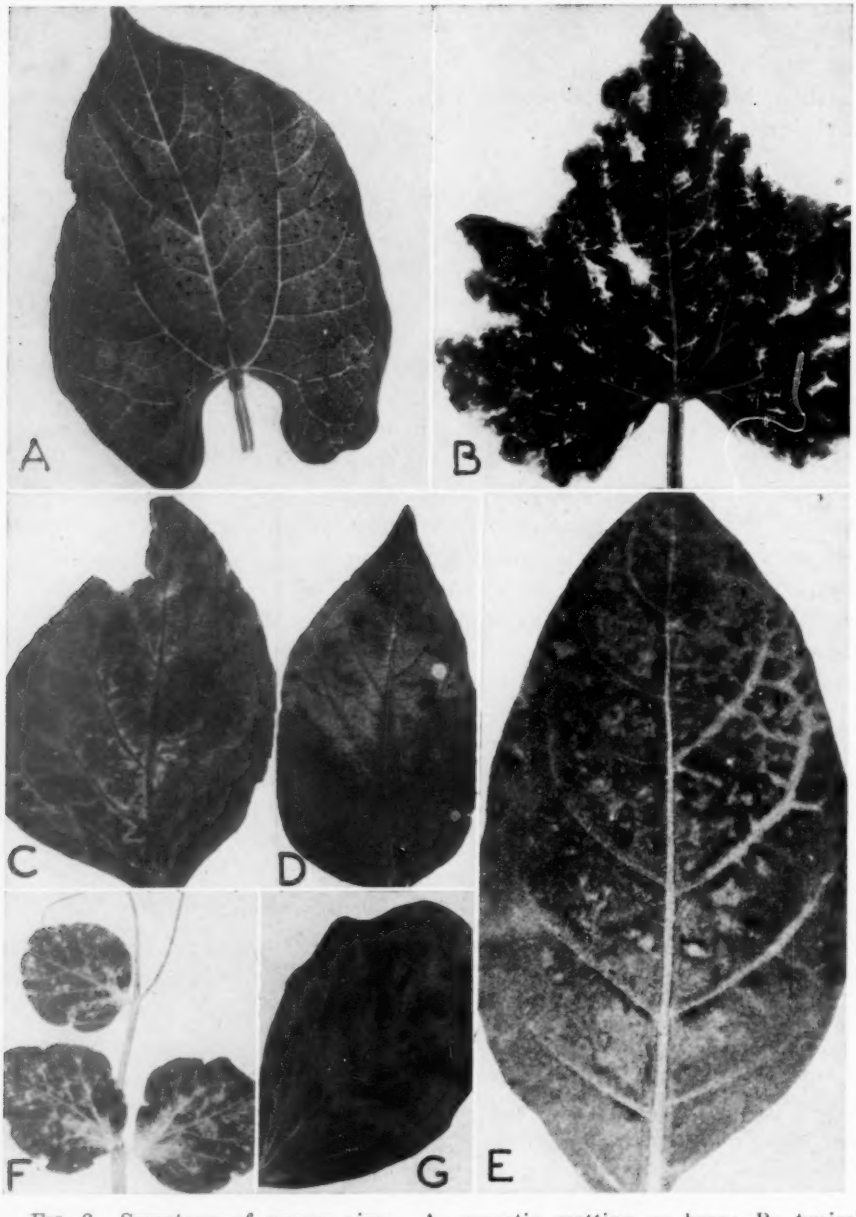

FIG. 2. Symptoms of pepper virus. A: necrotic spotting on bean; B: typical chlorotic vein-banding type of mottle on cucumber; C: chlorotic mottle and patterns on pepper; F: mottle on pea; G: necrotic lesions on broad bean. D and E: symptoms of potato-calico virus on pepper and *Nicotiana rustica*. Note wide vein-banding on *N. rustica* and lack of chlorotic patterns on pepper. The three spots in D were caused by film defects and are not symptoms of virus infection.

vein-banding, chlorotic mottle, chlorotic rings, necrotic flecks, and necrotic line patterns associated with the larger veins.

Snap bean var. Stringless Evergreen. Four series of inoculations, involving 20 plants, were carried out on this host. Only primary symptoms were obtained, consisting of small dark necrotic lesions (Fig. 2, A).

Windsor Broad Bean. Four series of inoculations, involving 20 plants, were effected. Symptoms consisted of black primary necrotic lesions followed by systemic necrosis and necrotic spotting (Fig. 2, G) sometimes resulting in the death of the plants. A mild chlorotic mottle was present in some cases where the necrosis was not severe.

Soybean var. Blackeye. Three series of inoculations, involving 10 plants, were carried out. All inoculated plants developed a mild systemic mottle, with no primary symptoms.

Datura stramonium. On this host, five series of inoculations, involving 25 plants, were effected. Symptoms consisted of primary yellow lesions, followed by systemic mild mottle with fine grayish-white etched rings and sometimes mild vein-banding.

Field pea var. Laxton's Progress. Twelve series of inoculations, involving 58 plants, gave somewhat inconsistent results in that the percentage of successful inoculations was at times very low. At other times infection was one hundred per cent. The first symptom was a drying out of the rubbed leaves. This was followed by a general wilting and stunting of the plant, with mild mottling of tip leaves (Fig. 2, F). In some cases, only tip leaves wilted, while in others, the plants were killed. The foliage became light green to yellow and the general appearance of affected plants suggested root injury rather than symptoms resulting from virus infection. Accordingly, transfers were made from wilted tip leaves of pea to *Nicotiana tabacum* and *N. glutinosa* resulting in symptoms typical for the pepper virus, thus indicating that these unusual symptoms on pea were due to infection by the virus.

Sweet pea var. Pinkie. Eleven series of inoculations, involving 43 plants, were carried out on sweet pea. Primary symptoms consisted of solid necrotic "tear drop" lesions on the rubbed leaves, which later were cast (Fig. 1, E). Systemic symptoms, which were not general on a plant, comprised curling and dwarfing of tip leaves, while only a few leaves developed chlorotic spotting or white necrotic streaks or stippling, generally towards the base of affected leaves (Fig. 1, F). All plants were stunted and, in some cases, were killed. Often one lateral shoot was killed while the remainder of the plant continued to grow, though growth was severely stunted.

Red clover. On this host seven series of inoculations, comprising 32 plants, gave positive results in only 3 series. Accordingly, it would appear that red clover is not readily infected by the pepper virus. Symptoms, when obtained, consisted of general mottling with slight distortion of leaves. In one series large brown necrotic lesions developed on the rubbed leaves.

Cucumber var. Windermoor Wonder. Fourteen series of inoculations, comprising 52 plants, gave inconsistent results in that in six series negative results were secured. In eight series, however, symptoms consisting of general mottling of a chlorotic vein-banding and asteroid-like type were consistently obtained (Fig. 2, B). Affected plants became stunted and the fruit showed chlorotic spots and rings with little or no distortion.

Pepper var. California Wonder. Only 4 series of inoculations, involving 24 plants, were tested on this host. All plants showed similar symptoms, consisting of general mottle with chlorotic rings, spots, and often chlorotic line patterns (Fig. 2, C).

Snapdragon var. Empress. In this case also only 4 series of inoculations, comprising 22 plants, were tested. Symptoms consisted of reddish rings or patterns on rubbed leaves, and sometimes were more readily apparent on the lower than the upper surface of the leaf. Transfers from such leaves to *Nicotiana tabacum* and *N. glutinosa* produced symptoms on these hosts typical for the pepper virus. No definite systemic symptoms were apparent.

Tomato var. Grand Rapids. Inoculations in six series, and involving 28 plants, gave negative results. Inoculations from tomato back to *Nicotiana tabacum* or *N. glutinosa* gave no symptoms, indicating that the tomato plants were not acting as "carriers" of the virus. It would appear therefore that tomato is not a host of the pepper virus.

Petunia. The symptoms on petunia consisted of dark green rings on rubbed leaves and systemic vein-clearing or general vein-banding on the upper leaves.

Zinnia. In eight series of inoculations, comprising 34 plants, rather inconsistent symptoms were obtained. The first sign of infection was the turning downward of certain leaves, or a temporary twisting of the tip of the plant. Primary symptoms consisting of yellow lesions were sometimes present, sometimes absent. Systemic mottling was largely confined to the newer leaves. In many cases the mottle was indistinct, while in others it was definite and of the spot type.

Celery var. Golden Plume. On celery five series of inoculations, involving 20 plants, were effected. A chlorotic mottle, especially on the older leaves, general stunting, and mild distortion of leaflets were the only symptoms. The chlorotic areas were usually more numerous towards the margins of the leaf. In time much of the chlorotic tissue faded to almost white.

Eggplant, var. Black Beauty. Only a few inoculations were made on eggplant but all produced a general systemic mottle.

COMPARISON OF THE SYMPTOMS CAUSED BY THE PEPPER VIRUS WITH THOSE CAUSED BY THE ALFALFA-MOSAIC VIRUS AND THE POTATO-CALICO STRAIN

The same number of series of inoculations, as effected for the pepper virus, were also carried out on the same hosts with *Marmor medicaginis* var.

typicum and *M. medicaginis* var. *solani*. This was necessary since many of the descriptions of symptoms given in the literature for alfalfa mosaic and potato calico are meagre and insufficient for comparative purposes.

In general, it was found that the alfalfa-mosaic and potato-calico viruses produced symptoms of the same general type as those associated with the pepper virus. For instance, on tobacco, broad bean, bean, sweet pea, pea, soybean, *Datura stramonium*, snapdragon, eggplant, petunia, celery, and zinnia, all three viruses produced similar symptoms, differing at times in degree only. However, the symptoms on certain hosts differed to a sufficient degree to suggest that the potato-calico virus and the pepper virus should be considered as distinct strains of the alfalfa-mosaic virus.

The pepper virus produced more necrosis on *Nicotiana tabacum*, *N. glutinosa*, and *N. rustica* than did either the alfalfa-mosaic virus or the potato-calico virus. In this respect, the alfalfa-mosaic virus was intermediate between the pepper virus and the potato-calico virus, which produced the least necrosis. The pepper virus did not produce symptoms on tomato, whereas this host was readily infected by both the alfalfa-mosaic and the potato-calico viruses. The potato-calico virus produced more vein-clearing and fine vein-banding than either of the other viruses. On red clover both the alfalfa-mosaic and pepper viruses produced mottling with distortion of leaves, whereas distortion was, for the most part, lacking with the potato-calico virus.

The potato-calico virus caused a pronounced blotchy mottle on pepper (Fig. 2, D), whereas, with the alfalfa-mosaic and pepper viruses, the mottle was finer and chlorotic rings were more common. Whereas the potato-calico virus produced a coarse blotchy mottle with prominent wide vein-banding on *Nicotiana glutinosa* and *N. rustica* (Fig. 2, E), the alfalfa-mosaic virus and the pepper virus produced only a chlorotic mottle with rings, lines, etc.

No infection was obtained on cucumber with the potato-calico virus whereas this host was readily infected by the alfalfa-mosaic and pepper viruses.

As to necrosis on broad bean it was least with the potato-calico virus and greatest with the pepper virus.

CROSS PROTECTION TESTS

To verify further the close relationship of these viruses, *Nicotiana tabacum* plants systemically infected with Nicotiana virus 1, Cucumis virus 1, alfalfa-mosaic virus, and the potato-calico virus were re-inoculated with the pepper virus by rubbing one half of two or three leaves on each plant. No symptoms developed on the rubbed portions of leaves on the plants previously infected with the alfalfa-mosaic virus or the potato-calico virus, but typical local lesions and chlorosis developed on the rubbed leaves of the plants systemically infected with Nicotiana virus 1 and Cucumis virus 1. These results indicate that the pepper virus is closely related to the alfalfa-mosaic virus.

THERMAL DEATH POINT

The thermal death point of the pepper virus was between 63° C. and 64° C. Some tests at 62° C. were positive and some negative. All tests at 61° C. were positive while at 63° C. and 64° C. all were negative.

According to Pierce (4) and Zaumeyer and Wade (10), the thermal death point of alfalfa virus 1 is between 62° C. and 65° C., while Zaumeyer (8) found it to be between 65° C. and 70° C.

Therefore the thermal death points for the alfalfa-mosaic virus and the pepper virus are in close agreement.

DISCUSSION

The symptoms produced by the pepper virus on 17 of the 18 hosts used in this investigation were in close agreement with symptoms associated with the alfalfa-mosaic virus on the same hosts. Moreover, the cross protection and thermal death point tests also indicated that the pepper virus was closely related to the alfalfa-mosaic virus. However, the fact that the pepper virus did not infect tomato and that it consistently produced more necrosis than the alfalfa-mosaic virus, would suggest that the pepper virus should be considered to be a strain of the alfalfa-mosaic virus.

The cross protection tests also indicated that the potato-calico virus was closely related to the alfalfa-mosaic virus. However, the negative results on cucumber, and the slightly different symptoms produced by the potato-calico virus on *Nicotiana glutinosa*, *N. rustica*, and pepper in comparison with those associated with the alfalfa-mosaic virus and the pepper virus on these same hosts, would suggest that the potato-calico virus also should be considered a distinct strain of alfalfa-mosaic virus. These results are, therefore, in agreement with those of Black and Price (1), who named the potato-calico virus *Marmor medicaginis* var. *solani*.

It is suggested that the pepper virus be called *Marmor medicaginis* var. *capsici*.

SUMMARY

An unusual type of mosaic was found on sweet pepper var. California Wonder in Ontario in 1943 and again in 1944. Symptoms included chlorotic rings, spots, and patterns on both leaves and fruit.

A large number of inoculations, extending over a 3-year period, using *Marmor medicaginis* H. var. *typicum*, *M. medicaginis* H. var. *solani* and the pepper virus indicated that the pepper virus was a strain of *Marmor medicaginis*. Comparative studies were made on *Nicotiana tabacum* L.; *N. rustica* L.; *N. glutinosa* L.; *Vicia faba* L., broad bean; *Lathyrus odoratus* L., sweet pea; *Pisum sativum* L., pea; *Phaseolus vulgaris* L., bean; *Trifolium pratense* L., red clover; *Capsicum frutescens* L., pepper; *Soja max* (L.) Piper, soybean; *Solanum Melongena* L., eggplant; *Zinnia elegans* Jacq., zinnia; *Cucumis sativus* L., cucumber; *Petunia hybrida* Viln., petunia; *Lycopersicon esculentum* Mill., tomato; *Antirrhinum majus* L., snapdragon; and *Apium graveolens* L., celery.

With the following exceptions, all 3 viruses produced similar symptoms on the above hosts. The pepper virus did not infect tomato, while both the alfalfa-mosaic virus and the potato-calico strain did. Whereas, the pepper virus and the alfalfa-mosaic virus gave positive results on cucumber, negative results were obtained with the potato-calico virus. The potato-calico strain produced prominent calico symptoms with wide vein-banding on *Nicotiana rustica* and *N. glutinosa*. On these same hosts, alfalfa-mosaic and pepper viruses produced much less prominent symptoms, with the wide vein-banding lacking. The pepper virus produced more severe necrosis on *N. glutinosa*, *N. rustica*, and *N. tabacum* than did the alfalfa-mosaic virus or the potato-calico virus.

Cross protection and thermal death point tests also indicated that the pepper virus was a strain of *Marmor medicaginis* H.

It is suggested that the pepper virus be called *Marmor medicaginis* var. *capsici*.

DOMINION LABORATORY OF PLANT PATHOLOGY,
ST. CATHARINES, ONTARIO.

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METHODS FOR DETERMINING RESISTANCE OF OATS TO HELMINTHOSPORIUM VICTORIAE[1]

S. C. Litzenberger and H. C. Murphy[2]

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INTRODUCTION

Helminthosporium blight of oats caused by *Helminthosporium victoriae* Meehan and Murphy[3,4,5,6] was first observed in the field in 1945. In 1946, it had become a major disease of oats in Iowa, Illinois, Indiana, New York, and certain other states. A large portion of the oat acreage in the United States was sown to crown rust resistant varieties that had been derived from Victoria (C.I. 2401[7]), a variety introduced from South America in 1927. Susceptibility to *H. victoriae* has been inherited with the "Victoria type" of resistance to *Puccinia coronata* Corda (Fig. 1). While the new crown rust resistant derivatives from Victoria (Boone, Tama, Control, Cedar, Vicland, Vikota, Osage, Neosho, Ventura, Forvic, Letoria, Victorgrain, Fulgrain, Stanton and others) were being developed and released, the helminthosporium blight was unknown and apparently of little or no importance as these varieties were consistently high in yield because of their relative freedom from disease, particularly crown rust. With the sudden appearance of this new disease in the field in 1945, however, and its rapid development to epiphytotic proportions on the Victoria derivatives in certain areas in 1946, the need for further study became imperative.

Although there were available certain methods for inoculating oats to determine their susceptibility to *Helminthosporium victoriae* in the greenhouse, they were not feasible for large scale use in the field. The relative effectiveness of several techniques applicable to large-scale evaluation in

[1] Journal Paper No. J–1438 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 72. Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, United States Department of Agriculture, and the Botany and Plant Pathology Section of the Iowa Agricultural Experiment Station.

[2] Industrial Fellow, Botany and Plant Pathology Section, Iowa Agricultural Experiment Station and Senior Pathologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, United States Department of Agriculture. The writers wish to express their appreciation to Dr. A. G. Johnson for valuable suggestions in the preparation of the manuscript, to Miss Frances Meehan and Mr. Darrell Morey for assistance during the investigation, to Mr. John Staby for photographic work, and to the Quaker Oats Company for grant of funds for part of this study.

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the greenhouse and in the field were therefore compared and the results are herein reported.

MATERIALS AND METHODS

During early spring and autumn of 1946, investigations were conducted in the greenhouse and in nursery at the Agronomy Farm at Ames, Iowa, to determine the relative effectiveness of ground, infected straw, naturally infested soil, and macerated cultures of *Helminthosporium victoriae* in inducing infection on susceptible and resistant oat varieties.

The infected straw was of susceptible varieties grown in nursery experiments either at the Agronomy Farm or at the Ash Avenue Farm at Ames,

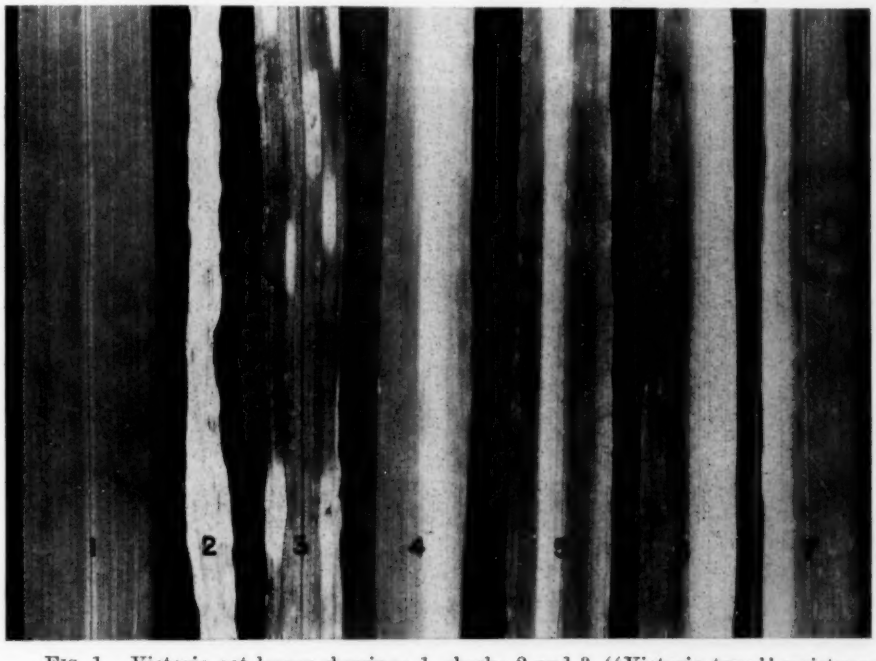

FIG. 1. Victoria oat leaves showing: 1, check; 2 and 3, "Victoria type" resistance to crown rust, the latter showing individual points of infection; and 4 to 7, characteristic striping from helminthosporium blight.

Iowa. The straw from the Agronomy Farm was naturally infected, while that of the Tama plants at the Ash Avenue Farm had been artificially inoculated with a spray of mycelial suspension just before jointing, during a cloudy, rainy period. The darkened nodes and stems of the straw, which contained abundant conidia of the fungus, were ground in a hammermill to a size that would pass through openings about 1 mm. in diameter. In all the trials involving ground straw one teaspoonful of such material was added to each 4-inch pot or 5-foot nursery row. The naturally infested soil was from the Agronomy Farm where badly infected oats had been grown the previous crop season.

For the experiments with artificial cultures of *Helminthosporium vic-*

toriae, the following three cultures were used: (1) The original isolate (H96) and (2) a sporulating strain (H224), both isolated from oats at Ames, Iowa; and (3) an isolate from oats (designated H.R.R.) received from Dr. H. R. Rosen, Fayetteville, Arkansas. These cultures were grown on oatmeal agar in 100 × 15 mm. Petri dishes, each containing about 15 cc. agar, for at least ten days at room temperature, and then macerated for 45 seconds in a Waring Blendor. Ten Petri-dish cultures, with or without the agar, that is, in the latter, the culture scraped from the agar, were added to 500 cc. distilled water in the blender. This, when macerated, constituted the "basic suspension." This basic suspension (designated B.S.) and also dilutions of it, one part basic suspension to one part distilled water (designated "50 per cent B.S.") or one part basic suspension to 19 parts distilled water (designated "5 per cent B.S."), were used as inoculum. Unless

FIG. 2. Relative susceptibility of: 1, Victoria, 2, Vicland, and 3, Bond, when sprayed with *Helminthosporium victoriae,* basic suspension without agar.

otherwise stated, these suspensions were applied at the rate of 50 cc. for each 4-inch pot. When the plants were sprayed with any suspension, the leaves were previously rubbed gently to remove most of the waxy covering.

In the greenhouse experiments, 25 to 50 seeds were sown in each 4-inch pot, while in the field experiments, 100 seeds were planted in each 5-foot nursery row. All treatments were replicated three times except in one experiment where the resistant variety was replicated only once. The experimental design of all tests was a randomized block.

Except where naturally infested soil was used, steamed soil, consisting of equal parts of compost, sand, and Clarion loam, was used in the greenhouse experiments. Temperatures in the greenhouse for the duration of most experiments ranged from 65° to 90° F., except in the temperature-controlled experiments.

RESULTS OF GREENHOUSE EXPERIMENTS

After preliminary tests had been made in the late winter of 1946 on several different methods of inducing infection of oats with *Helminthosporium victoriae,* seven series of experiments were conducted in the greenhouse at 50° F. and 80° F. with the most promising methods. These included the use of ground infected straw and stubble, infested soil, and the fungus grown artificially on oatmeal agar, and handled in various ways. Some of the typical data on emergence and survival are given in table 1 and illustrated in figures 2 and 3.

No plants of susceptible varieties survived when the basic suspension of either dilution, either with or without the agar, was used as inoculum. The treatments were equally effective when the suspension was added to the seed at sowing time or applied as a spray on the young oat seedlings and incubated in a moist chamber for 36 hours. Usually comparable results were obtained from the basic suspensions and the 50 per cent and the 5 per cent dilutions from them, respectively, whether they were applied with the seed at sowing or as a spray on the seedlings.

No significant differences were observed between the use of cultures removed from the agar and those used together with the agar in preparing the basic suspensions. Both methods were effective in eliminating all plants of susceptible varieties. The basic suspension with the agar, applied directly to the seed, dried, and stored at room temperature for various periods up to more than four months also was effective. As will be pointed out in the following section, this method affords an excellent means of handling oat material for field or nursery plantings.

When ground infected straw was used as a source of inoculum (Series A, 2 and 3) a few of the susceptible plants escaped being killed when the straw was added to the seed at the time of sowing. These surviving plants were considerably etiolated and less than one-half the height of plants grown in sterile soil. It is doubtful that these plants would have produced seed if allowed to continue development. When the infected straw was applied broadcast on previously rubbed and wetted plants, however, or applied to the surface of the soil after the oat plants had emerged and the potted plants placed in the moist chamber for 36 hours, the infected straw was as effective as the basic suspension for obtaining complete infection in susceptible varieties. When infected straw is used as a source of inoculum, apparently incubation in the moist chamber is necessary for complete elimination of the susceptible plants. Otherwise escapes may occur.

Soil infested with *Helminthosporium victoriae* did not prove entirely satisfactory for inducing uniform infection in susceptible varieties in the greenhouse, as some plants escaped attack (Series A, 9–10). Where sharp differences between resistance and susceptibility are required, this method is not so satisfactory as the others that eliminate the susceptible plants completely.

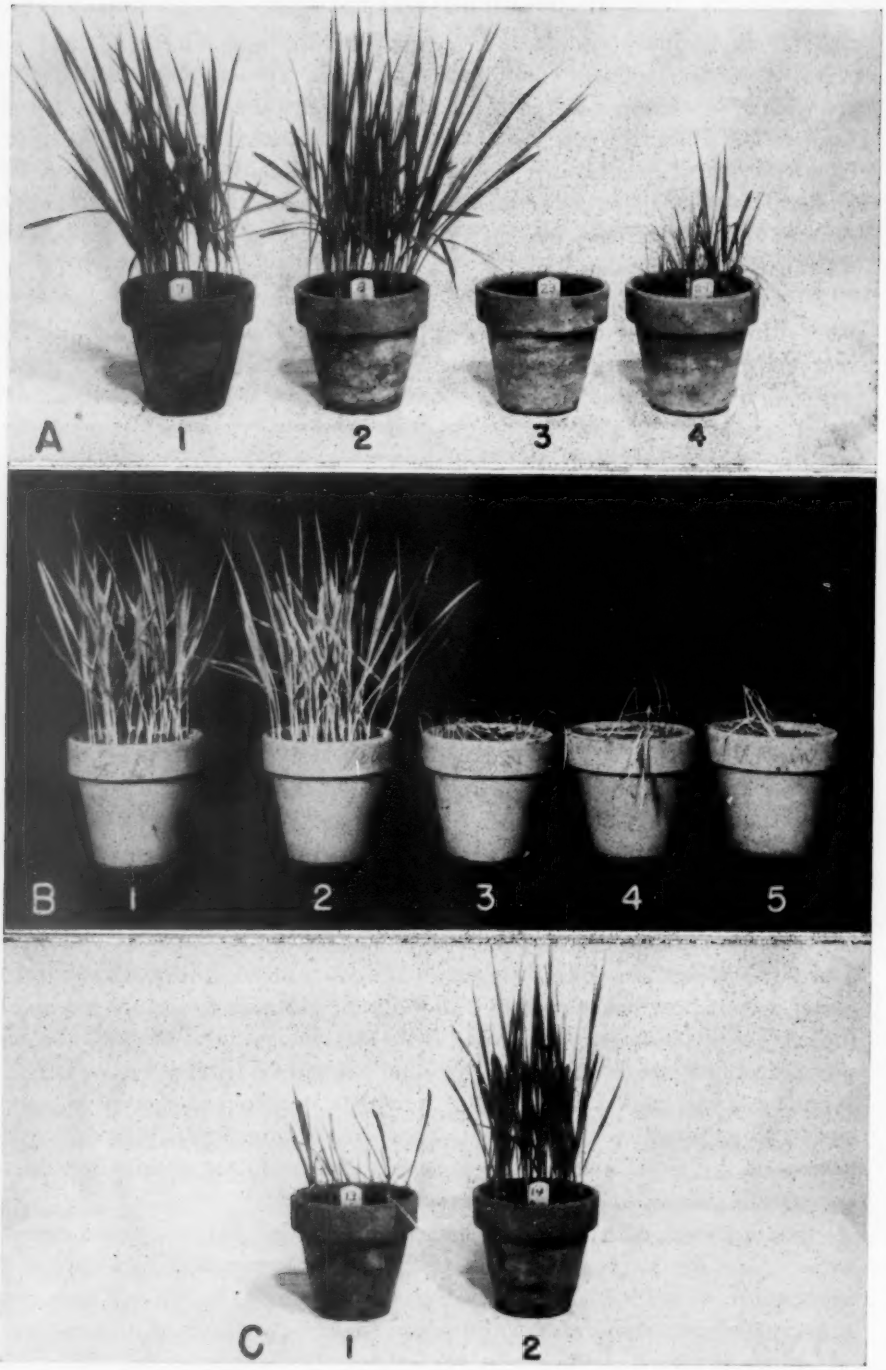

FIG. 3. Clinton, Vicland, and Cedar oat plants grown in the greenhouse from seed noninoculated or variously inoculated with *Helminthosporium victoriae* at sowing or

All emerged plants of resistant varieties continued to develop normally while infected plants of susceptible varieties rarely developed beyond the primary leaf stage. The emergence of susceptible varieties varied, with the method of inoculation employed, from zero to nearly 100 per cent. The lowest emergence counts were obtained at the higher temperature (80° F.). especially when the seed had been inoculated with basic or 5 per cent basic suspension (Series A, 15 and 16; Series F, 1 and 2). The lower temperature (50° F.) was somewhat less favorable for the fungus than the higher temperature and consequently percentage emergence usually was lower at the higher temperature. At both temperatures, however, the percentage survival was uniformly zero. With the resistant variety, Clinton, emergence was depressed comparatively little by the inoculation. In only one case (Series A, 15), was it depressed to as low as 64 per cent. In this case the basic suspension had been used with the seed at sowing. Shallower seedings and the use of more dilute suspension should overcome any significant emergence depression that may otherwise occur in resistant varieties.

In some cases (Series E, 4) the use of the macerated sterile oatmeal agar alone delayed germination of both susceptible and resistant oat varieties as well as reduced emergence. In other cases (Series F, 5) no significant effects were apparent.

In no case did the resistant varieties included in this test succumb to or show any symptoms of being infected by *Helminthosporium victoriae*. Seed that failed to germinate or seedlings that failed to emerge showed no evidence of infection. Only those varieties that have the crown rust resistance of Victoria were found susceptible. In fact, this was one of the methods used to determine whether surviving plants were escapes or mixtures. The surviving plants were inoculated with crown rust race 45. If susceptible they were classed as mixtures and if having the Victoria type of crown rust resistance, they were classed as escapes. The final check for determining escapes, however, was by spraying the plants with a mycelial suspension of *H. victoriae*.

The above results indicated that resistance or susceptibility of oats to *Helminthosporium victoriae* could be accurately and readily determined in the greenhouse by inoculating with infested straw or especially with suspensions of cultures of the fungus either with or without the agar.

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before, when so stated: A, 1, Clinton inoculated with culture H96, basic suspension (= B.S.), with agar; A, 2, Clinton inoculated with H96, 5 per cent B.S., with agar; A, 3, Vicland inoculated with H96, B.S., with agar; A, 4, Vicland inoculated with H96, 5 per cent B.S., with agar; B, 1, Clinton not inoculated (control); B, 2, Vicland not inoculated (control); B, 3, Vicland inoculated with H224, 5 per cent B.S., with agar; B, 4, Vicland inoculated with H96, 5 per cent B.S., with agar; B, 5, Vicland, inoculated with culture H.R.R. from Arkansas, 5 per cent B.S., with agar; C, 1, Cedar, seed moistened with B.S. of H96, incubated 20 hours at room temperature and stored 4 months before sowing; C, 2, Cedar not inoculated (control).

TABLE 1.—*Emergence and survival of Clinton (Resistant) and Victoria, Vicland, and Cedar (Susceptible) oats following various methods of inoculation with Helminthosporium victoriae in the greenhouse at 50° and 80° F., Ames, Iowa, 1946*

Series and unit No.	Inoculum[a] and method[b] of inoculation	Resistant variety		Susceptible variety			
		50° F.	80° F.	50° F.		80° F.	
		Emerg.[c]	Emerg.[c]	Emerg.	Surv.	Emerg.	Surv.
		Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
		Clinton				*Victoria*	
A, 1	None (Control)	.....	96	89	100	83	100
						Vicland	
D, 7	Do	98	90	100	100	96	100
, 8	None (Seed treated)[d]	100	100	100	100	100	100
E, 4	With sterile agar, with seed at sowing[e]	.....	95	.....	.....	85	100
, 5	None (Control)	.....	100	.....	.....	99	100
F, 5	With sterile agar, with seed at sowing[e]	98	100	.....	.....	.....	.....
, 6	None (Control)	100	97	.....	.....	.....	.....
, 7	None (Seed treated)[d]	100	100	.....	.....	.....	.....
	Culture unidentified					*Victoria*	
A, 2	Ground straw, with seed at sowing	.....	88	64	0	57	4
, 3	Ground stubble, do	.....	88	80	7	79	13
, 4	Ground straw, on soil surface when plants emerged	.....	96	.....	.....	96	0
, 5	Ground stubble, do	.....	92	.....	.....	91	3
, 6	Ground straw, on emerged plants	.....	96	.....	.....	94	0
, 7	Ground stubble, do	.....	92	.....	.....	92	0
, 8	Ground straw, on soil surface at sowing	.....	88	.....	.....	87	0
, 9	Infested soil I	.....	88	80	0	83	2
, 10	Infested soil II	.....	96	75	8	79	8
	Culture H96 (Basic suspension)						
A, 11	Without agar, sprayed on seedlings	.....	92	.....	.....	88	0
, 13	With agar, do	.....	92	.....	.....	89	0
, 15	Without agar, with seed at sowing	.....	64	.....	0	3	0
						Vicland	
E, 3	Do	.....	96	.....	.....	.....	.....
F, 1	Do	93	87	15	0	12	0
D, 2	With agar, with seed at sowing	86	96	50	0	10	0
E, 2	Do	.....	73	.....	.....	50	0
F, 3	Do	.....	82	.....	.....	0	0
						Victoria	
A, 17	Do	.....	80	.....	0	21	0
						Cedar	
B, 1	With agar, on seed, stored 0 days	.....	.....	.....	.....	40	0
C, 1	Do 60	.....	.....	.....	.....	64	0
G, 1	Do 90	.....	.....	.....	.....	68	0
D, 1	Do 120	.....	.....	.....	.....	72	0
E, 1	Do 130	.....	.....	.....	.....	44	0

TABLE 1—(*Continued*)

Series and unit No.	Inoculum[a] and method[b] of inoculation	Resistant variety		Susceptible variety			
		50° F.	80° F.	50° F.		80° F.	
		Emerg.[c]	Emerg.[c]	Emerg.	Surv.	Emerg.	Surv.
		Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
	(50 per cent basic suspension)		*Clinton*			*Vicland*	
D, 5	Without agar, with seed at sowing	100	94	76	0	36	0
, 3	With agar, do	96	96	68	0	26	0
	(5 per cent basic suspension)					*Victoria*	
A, 12	Without agar, sprayed on seedlings	.....	92	.....	.....	88	0
, 14	With agar, do	.....	96	.....	.....	88	0
, 16	Without agar, with seed at sowing	.....	76	.....	0	8	0
						Vicland	
D, 6	Do	100	98	92	0	68	0
F, 2	Do	100	96	40	0	17	0
D, 4	With agar, with seed at sowing	100	99	93	0	82	0
F, 4	Do	.....	97	.....	.....	21	0
						Victoria	
A, 18	Do	.....	96	.....	0	32	0
	Culture H.R.R.						
	(50 per cent basic suspension)					*Vicland*	
D, 9	Without agar, with seed at sowing	100	96	66	0	46	0
	(5 per cent basic suspension)						
D, 10	Without agar, with seed at sowing	100	100	84	0	92	0
	Culture H224						
	(Basic suspension)						
C, 2	Without agar, sprayed on seedlings	.....	.....	.....	.....	.....	0
	(50 per cent basic suspension)						
D, 11	Without agar, with seed at sowing	100	98	100	0	86	0
, 13	With agar, do	100	98	86	0	78	0
	(5 per cent basic strength)						
D, 12	Without agar, with seed at sowing	100	100	92	0	90	0
, 14	With agar, do	98	100	92	0	84	0

[a] Ground straw inoculum was from Ash Avenue Farm; ground stubble, from Agronomy Farm. Infested soils I and II were from Agronomy Farm.

[b] When straw or stubble inoculum was placed on surface of the soil after plants emerged, or on emerged plants, the plants were placed in a moist chamber for 36 hours after inoculation. When seed was inoculated and stored before planting, the seed was moistened with the basic suspension, incubated 20 hours, then dried and stored.

[c] In all cases 100 per cent of the resistant Clinton plants survived.

[d] Seed in Series D, 8 and F, 7 was treated with DuBay 1452–F (6.5 per cent ethyl mercuri-p-toluene sulfonanilide).

[e] The sterile oatmeal agar was applied at the same rate as the treatment with viable basic suspension.

RESULTS OF NURSERY EXPERIMENTS

Since the greenhouse experiments had indicated that some of the methods of inoculation might be satisfactory for use in the field, six methods of inoculation, four with infected straw and two with basic suspension, together with a noninoculated control, were included in an experiment in the field on summer fallow. Although seeded in early September, 1946, the weather that followed was comparable to that usually prevailing in the spring season. The resulting data on survival are given in table 2.

TABLE 2.—*Percentage survival of plants of susceptible*[a] *Vicland and Cedar oats following different methods of inoculation with Helminthosporium victoriae in the field at Ames, Iowa, 1946*

Treatment No.	Inoculum and method of inoculation	Percentage plants surviving			
		Vicland	Cedar		Ave.
		I	I	II	
1	None (Control)	100	100	100	100
2	Ground straw, on soil surface	6	2	46	18
3	Do , with seed[b]	6	4	20	10
4	Do , do	0	0	6	2
5	Do , moistened on seed and incubated for 20 hours before sowing	2	0	7	3
6	Culture H96, with agar (B.S.[c]), do	0	0	0	0
7	Do , moistened on seed and dried immediately before sowing	0	0	0	0

[a] Resistant Bond and Clinton varieties were also included in the experiment, but no differences in survival were observed with any of the treatments. All plants survived 100 per cent.

[b] Seed was sown ½ inch deep in treatment no. 3 and 1½ inches deep in all of the others.

[c] B. S. indicates basic suspension.

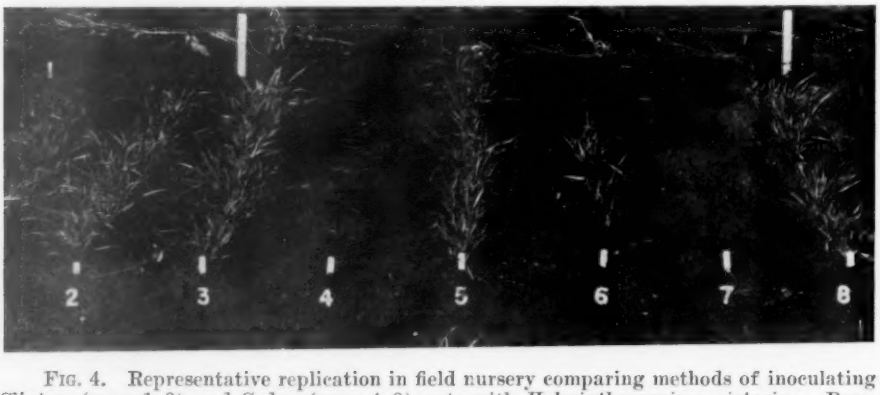

FIG. 4. Representative replication in field nursery comparing methods of inoculating Clinton (rows 1–3) and Cedar (rows 4–8) oats with *Helminthosporium victoriae*. Rows 1 and 7 (=treatment 6, table 2), seed moistened with basic suspension and incubated 20 hours before sowing; rows 2 and 8 (=treatment 2, table 2), ground infected straw on surface of soil after seeding; rows 3 and 5 (=treatment 1, table 2), noninoculated controls; row 4 (=treatment 7, table 2), seed moistened with basic suspension and dried immediately; row 6 (=treatment 4, table 2), seed dusted with ground, infected straw and incubated 20 hours at room temperature before sowing.

The only two methods of inoculation that were uniformly effective in eliminating all plants of susceptible varieties were those used in series 6 (Fig. 4, row 7) and series 7 (Fig. 4, row 4). In both of these, the basic suspension of cultures had been applied to the seed before sowing: Series 6, incubated 20 hours at room temperature, and series 7, dried immediately after inoculation. The results from the use of ground infected straw were erratic, because from 2 to 18 per cent of the susceptible plants escaped the attack of *Helminthosporium victoriae*. Placing the infected straw with the seed at sowing time at a depth of 1½ inches was more satisfactory than the shallower seedings.

No loss of plants in the resistant varieties was apparent, indicating that the more effective methods can be satisfactorily used under nursery or field conditions to determine varietal reaction to this disease.

SUMMARY

Methods of inoculating oats with *Helminthosporium victoriae* under greenhouse and nursery conditions were compared at Ames, Iowa, in 1946. Mycelial suspensions of the organism were found most uniformly satisfactory in inducing heavy infections in susceptible varieties of oats. The basic suspensions and also 50 per cent and 5 per cent dilutions of them, with or without the agar, applied with the seed at sowing, on the surface of the soil after emergence, or as a spray on the seedlings after emergence, gave no survival in susceptible varieties, while corresponding plants of resistant Clinton were not significantly injured. When the suspensions were applied on the surface of the soil after emergence or as a spray on the plants, incubation in a moist chamber for 36 hours was necessary.

Seed moistened with the basic suspension, dried immediately or incubated at room temperature for 20 hours, planted immediately or stored for various periods up to more than 4 months also gave equally satisfactory results in both greenhouse and nursery experiments.

In the greenhouse, when *Helminthosporium*-infected straw was applied broadcast to the soil surface or on moistened emerged plants at the rate of one teaspoonful per pot and placed in the moist chamber for 36 hours no survival of susceptible plants was obtained. Dusting the seed with infested straw at seeding resulted in some susceptible plants surviving with every inoculation in the field and in the greenhouse experiments.

When sharp differences are necessary in a critical study of resistance or susceptibility of oats to this fungus, the method in which suspensions of cultures is used is preferred. Where large numbers of oat varieties or selections are to be tested under field conditions, however, and less critical differences are satisfactory, ground infected straw sown with the seed may be used.

In susceptible varieties emergence was reduced as much as 100 per cent by some methods of inoculation. In resistant varieties a slight depression

in emergence was observed, but all emerged plants continued to develop normally while infected plants of susceptible varieties rarely developed beyond the primary leaf stage. Germinated seeds of resistant varieties which did not emerge showed no evidence of infection. Percentage survival, therefore, is the best criterion for rating relative resistance of oat varieties to *Helminthosporium victoriae*.

AGRICULTURAL EXPERIMENT STATION,
AMES, IOWA.

TREATMENT OF LILY BULBS FOR BLACK SCALE CONTROL

F. J. LeBeau and F. J. Reynolds

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Black scale of Easter lily (*Lilium longiflorum*) was described by Plakidas[1] as causing severe losses to lily growers in Plaquemines Parish, Louisiana, as early as 1937. The disease increased and by 1943 there remained very few stocks of clean bulbs and few non-infested fields in the lily growing area. The increased value of lily bulbs arising from the curtailment of importation of this plant in 1941–42 stimulated a rapid expansion of lily bulb production in Terrebonne Parish, Louisiana, an area which had formerly produced few commercial bulbs. In the face of strong demand for large amounts of seed stock, little attention was paid to diseases, with the result that black scale as well as the virus diseases were introduced on the seed stock and widely distributed in Terrebonne Parish. Black scale became so threatening that in 1943, at the request of the lily growers in Terrebonne Parish, a rigid quarantine controlling the use of diseased stock was set up by the Louisiana State Department of Agriculture. While these measures reduced to some extent the further spread of the disease, black scale had become so well distributed before the quarantine that more adequate control measures were needed.

In preliminary bulb-treatment tests, Plakidas[1] used the common disinfectants available at that time. No one of these destroyed the pathogen in and on diseased bulbs or prevented infection of clean bulbs in the soil. Many of the fungicidal treatments were definitely injurious to the bulbs. In 1944, the senior writer took over the investigations and tested the more recently available organic fungicides.[2,3] The present paper reports the results of tests with a number of fungicides for controlling bulb-borne infection of *Colletotrichum lilii* and presents additional information on the use of Puratized N5E (phenyl mercuric triethanol-ammonium-lactate) for controlling black scale.

EXPERIMENTAL RESULTS

Most evaluations of the fungicides have been based on a laboratory test[2] which has given results that have agreed closely with those obtained in greenhouse and field tests. This test consisted of soaking detached infected scales in a fungicide for 24–48 hours and then soaking them in sterile water for 24 hours. After a 5-min. treatment in a saturated calcium hypochlorite solution, the scales were plated on potato-dextrose agar. In the more recent

[1] Plakidas, A. G. Black Scale: A disease of Easter Lily bulbs. Phytopath. **34**: 556–571. 1944.

[2] LeBeau, F. J. The eradicant action of a fungicide on *Colletotrichum lilii* in lily bulbs. Phytopath. **36**: 391–393. 1946.

[3] ———. A fungicide for protecting lily bulbs from infection by *Colletotrichum lilii*. Phytopath. **37**: 194–196. 1947.

work a wash in running tap water for 3–4 hours replaced the 24-hour soak in sterile water. Fungicide treatments were considered promising only if they eliminated *Colletotrichum lilii* and greatly reduced the other fungi associated with the *Colletotrichum*. When a treatment was ineffective *C. lilii* grew out from plated diseased scales and, in addition, a number of other fungi, principally species of *Fusarium* and *Penicillium*, appeared in the plates. Frequently the other fungi exceeded the *Colletotrichum*. Practically all lots of control scales, scales bearing black scale lesions and plated after a 5-min. treatment in calcium hypochlorite solution, have given rise to fungi. The fungus *Colletotrichum lilii* has appeared in approximately 50 per cent of the control scales, the incidence varying from 10 to 75 per cent in different test lots. Plakidas[1] noted that the ease of isolating *C. lilii* from diseased tissue varied from the extreme cases in which few cultures of *C. lilii* could be obtained from positively infected material to those in which 50 per cent or more of tissue platings yielded the pathogen. The ease with which *C. lilii* could be isolated varied with storage time after harvest, isolation being more difficult as storage time increased. In the present work scales stored for periods of 1–2 weeks to several months were used. In table 1, data on tests involving several materials are presented. Others tested, as Puratized 804 and IN 1452F (ethyl mercuric p-toluene sulfoanalide) at dilutions up to 1–4000, Dowicide 9B (zinc trichlorophenate) and New Im-

TABLE 1.—*The effects of fungicides on the survival of Colletotrichum lilii in infected lily scales*

Material[a]	Concentration[b]	Time (Hours)	Number of trials	Number of scales treated	Number of scales with	
					Total fungi[c]	*C. lilii*
Puratized N5E	1–1000	24	.....	150	0	0
Do	1–1000	24	.....	110	0	0
(without wetting agent)						
Puratized N5E	1–2000	48	.....	70	0	0
Do	1–8000	48	2	22	2	0
Puratized M. C.	1–1000	24	2	16	5	5
Isothan Q15	1–500	24	1	11	6	3
	1–1000	24	1	10	5	1
Dithane 14	1–100	24	1	9	.....	3
	1–1000	24	1	9	.....	4
8-Hydroxyquinoline sulphate	1–500	24	1	13	.....	5
Formalin, 5 per cent at 45° C.	.....	1	1	9	6	2
Control	.....	.....	.....	176	170	85

[a] Except where otherwise noted, IN 181P, a sodium lauryl wetting agent, was added at the rate of 1–4000. The Puratized compounds are phenyl mercuric triethanol-ammonium lactate; Isothan Q15 is lauryl isoquinolinium bromide; and Dithane 14 is a liquid disodium ethylene bisdithiocarbamate.

[b] Concentration based on the commercial product or experimental sample used and not on active ingredients. In all cases an excess of solution over that needed to cover the scales was used.

[c] Species of *Fusarium* and *Penicillium* were the predominant fungi appearing from plated scales.

proved Ceresan (ethyl mercuric phosphate) at dilutions up to 1–5000, and Dowicide A and B at dilutions up to 1–1000, were not included since injury to the scales was too severe for them to have any usefulness.

Only one material, Puratized N5E,[4] effectively destroyed all fungi occurring in infected scales without causing severe injury to healthy scales. When diseased lily scales were exposed to Puratized N5E, the infected areas were freed from all fungi and some of the healthy tissue surrounding the lesions was killed by the fungicide although healthy portions of the lily scale more distant from lesions were unaffected. When healthy lily scales were treated, the basal portions of the scales where they were broken from the bulbs were killed. Injury was also evident at points of other mechanical injury.

A wide range of concentrations of Puratized N5E was effective, depending upon the duration of treatment and the absolute amount of the toxicant in relation to the amount of plant material being treated. These relationships are illustrated by the data in table 2. A study of table 2 reveals that: (1) Regardless of concentration, a minimum time (about 24 hours) was required to completely eliminate *Colletotrichum lilii* from infected scales; (2) given the minimum effective time of treatment, an additional time, as was required for the more dilute solutions, merely served to compensate for the greater dilution rather than for the reduction in absolute amounts of the toxicant, since the combination of low concentration and long treatment was effective only when an excess amount of solution, in relation to the amount of scales being treated, was used; (3) in the range of concentration used, a second use of the same solution was effective only when an excess of solution over that required to cover the scales was used in the first treatment; (4) the most satisfactory combination of time and concentration from a practical standpoint is probably that of 24-hour treatment with a 1–1000 solution of Puratized N5E.

Other Factors Affecting the Use of Puratized N5E for Black Scale Control

Since it appeared that the long time needed for eradicating *Colletotrichum lilii* from diseased scales by Puratized N5E was required in order that the toxicant could penetrate the diseased tissues, the influence of reduced pressure on the effectiveness of the treatment was investigated. Infected scales in 1–500 and 1–1000 solutions of Puratized N5E were subjected to reduced pressure by the continuous operation of a vacuum pump, with an efficiency rating of 0.1 micron of mercury, for periods of 15 and 30 minutes, after which normal pressure was reestablished and the scales allowed to remain in the treating solution for such a time as to make the total time in the treating solution 1, 6, 12, and 24 hours. In no case was the 1-hour, and rarely was the 6-hour, treatment effective. In three sets of experiments

[4] Here Puratized N5E is used to include also Puratized N5X and Puratized Agricultural Spray, since each of these materials contains the same toxicant and when appropriately diluted has given similar results. The bulk of the data secured was with Puratized N5E.

involving 60 scales each, the 12-hour and the 24-hour treatments including either 15 or 30 minutes under reduced pressure completely eliminated all fungi. The use of reduced pressure was attended by greater injury than treatment at normal pressures. In one field test the reduction of pressure for 15 minutes with 6 hours total treating time (Table 5) caused a reduction in emergence. Only a fair degree of control was obtained.

TABLE 2.—*Relation of time, concentration, volume of treating solution in relation to volume of plant material, and successive use of the same solution on the effectiveness of Puratized N5E in eradicating Colletotrichum lilii from infected lily scales*

Concentration of Puratized N5E	Time (Hours)	Volume of solution	Times used	Number of scales plated	Number of scales with	
					Total fungi	*C. lilii*
1–500	6	excess[b]	1st	20	1	1
1–500	12	excess	do	20	1	1
1–500[a]	12	no excess[c]	do	30	0	0
1–500	24	excess	2nd	20	0	0
1–500	24	excess	1st	15	0	0
1–500	24	excess	2nd	15	0	0
1–500	24	no excess	1st	36	0	0
1–500	24	excess	2nd	20	4	2
1–1000	6	excess	1st	32	2	1
1–1000	12	excess	do	60	0	0
1–1000	12	no excess	do	32	3	1
1–1000	36	excess	2nd	26	3	2
1–1000	24	excess	1st	40	0	0
1–1000	24	excess	2nd	40	0	0
1–1000	24	no excess	1st	50	0	0
1–1000	24	excess	2nd	43	18	4
1–4000	12	excess	1st	40	17	7
1–4000	24	excess	do	40	1	0
1–4000	24	excess	2nd	20	2	0
1–4000	24	no excess	1st	55	4	3
1–4000	24	excess	2nd	45	28	15
1–4000	48	excess	1st	34	0	0
1–8000	24	excess	do	10	3	3
1–8000	24	no excess	do	30	6	6
1–8000	24	excess	2nd	26	21	9
1–8000	48	no excess	1st	36	12	4
1–8000	48	excess	do	22	2	0

[a] Indicates successive use of the same solution.
[b] An excess of solution was used over that required to cover the scales.
[c] Only enough solution to cover the scales was used.

Puratized N5E does not protect treated bulbs from reinfection when exposed to inoculum in infested soil, but, if after the Puratized N5E treatment, Arasan is dusted on the bulbs, both bulb-borne and soil-borne infections are controlled. Since the simultaneous application of both treatments would have a practical advantage, the effects of the presence of Arasan (tetramethyl-thiuram-disulfide), Tersan (similar to Arasan, but with a wetting agent), Fermate (ferric dimethyldithiocarbamate), Phygon (2,3,di-

chlor-1,4-naphthoquinone), and Yellow Cuprocide (yellow cuprous oxide) on the eradicant action of Puratized N5E were tested using the laboratory method of evaluation. These protective fungicides were added to 1–500 and 1–1000 solutions of Puratized N5E at the rate of one per cent of the protective fungicide and the suspensions obtained were used for treating infected lily scales. After treatment the scales were thoroughly washed in running tap water for several hours and after a 5-minute wash in calcium hypochlorite solution they were plated on potato-dextrose agar. The results of the test are shown in table 3.

TABLE 3.—*The effects of other fungicides added to Puratized N5E solutions on the eradicant action of Puratized N5E towards Colletotrichum lilii*

Concentration of Puratized N5E	Fungicide added	Number of scales treated	Number of scales with	
			Total fungi	*C. lilii*
1–500	Tersan	15	13	4
1–500	Fermate	15	14	3
1–500	Phygon	15	0	0
1–500	Yellow Cuprocide	15	0	0
1–500	None	15	0	0
1–1000	Tersan	15	12	2
1–1000	Fermate	15	15	3
1–1000	Phygon	30	4	0
1–1000	Yellow Cuprocide	15	0	0
1–1000	Arasan	15	12	.....
1–1000	None	30	0	0
None	Tersan	15	14	3
Do	Fermate	15	13	3
Do	Phygon	15	13	5
Do	Yellow Cuprocide	20	20	13
Do	Arasan	20	17	.....
Do	Water control	15	13	3

In these tests all of the fungicides derived from thiocarbamic acid interfered with the action of Puratized N5E when used simultaneously. Yellow Cuprocide had no effect while Phygon probably had only a slight inhibiting effect. None of the protective fungicides alone reduced to any appreciable extent the number of scales bearing *Colletotrichum lilii* or other fungi. Yellow Cuprocide seemed to have enhanced the ease with which *C. lilii* grew out from the scales.

Dupont IN 181P and Dupont IN 3622, detergents based upon sodium lauryl sulphate, were used as wetting agents in the Puratized N5E solutions, but no difference in the action of Puratized N5E was noted. These agents were used at the rate of 1–4000 in solutions of Puratized N5E varying in concentration from 1–500 to 1–4000 for time intervals of 12 to 48 hours.

Field and Greenhouse Studies

Only a few materials were tried in the greenhouse, and only the Puratized compounds were tested in the field. In the greenhouse tests, stem bulblets from a badly infected stock were treated and after treatment

planted in steamed Yohola silt loam which is the major soil type of the commercial lily area. Seven to eight months after planting, the bulbs were harvested for observation. Typical data from these tests are presented in table 4. Injury, evidenced by retarded and reduced emergence, was produced by treatments 1, 2, 3, 7, 8. The greatest injury was caused by treatment 1, in which 11 of the 15 bulbs dug had remained dormant throughout the period of the experiment. The New Improved Ceresan treatment retarded emergence as much as 6 weeks and frequently those plants which

TABLE 4.—*The effect of chemical treatment on the development of black scale on lily bulbs in steamed soil in the greenhouse. Bulblets from an infected stock were used*

	Test No. and Material	Concentration	Time (Hours)	Number of bulbs harvested	Number of clean bulbs	Number of diseased bulbs in each class[b]			Disease index[c]
						1	2	3	
1	IN 1452F	1–2000	48	15	12	3	0	0	7
2	Dithane	1–100	24	12	6	1	3	2	36
3	Dithane	1–50	24	33	15	7	3	8	38
4	Isothan Q15	1–125	24	13	6	4	1	2	31
5	Isothan Q15	1–250	24	18	11	2	0	5	26
6	Isothan Q15	1–1250	24	19	2	4	3	10	70
7	New Improved Ceresan	1–5000	24	14	9	2	2	1	22
8	New Improved Ceresan	1–5000	48	10	5	4	1	0	20
9	Puratized N5X	1–2000	48	22	22	0	0	0	0
10	do	1–500	24	17	16	1	0	0	2
11	do	1–1000	24	19	18	1	0	0	2
12	do	1–1000	6	19	12	2	1	4	28
13	do	1–2000[a]	48	7	0	2	2	3	71
14	Untreated	.....	.....	18	3	4	2	9	64

[a] Planted in non-steamed badly infested soil from Terrebonne Parish.

[b] 1 = Mildly diseased; 2 = moderately diseased; 3 = severely diseased.

[c] Clean, mild, moderately and severely diseased bulbs were given numerical values of 0, 33.3, 66.6 and 100 respectively. The disease index for any given treatment was obtained by summing the products of the number of bulbs in each class and the numerical value of the classes and dividing by the total number of bulbs.

emerged were malformed. The bulbs, however, were not destroyed, but persisted to the end of the test. While both New Improved Ceresan and IN 1452F reduced the incidence of black scale, both materials were inferior to Puratized N5X.

Puratized N5X almost completely prevented the development of black scale in all these tests when concentrations of 1–1000 to 1–2000 were used in 24- and 48-hour treatments. That Puratized N5X had no protective value is indicated by treatment 13.

The results of field tests on the use of Puratized N5X and N5E are given in table 5. Tests 2, 3, 4 were with commercial seed stock in which there was a high percentage of infected bulbs, the infection varying from mild to severe for individual bulbs. The over-all degree of infection was indicated as mild, moderate, and moderate for tests 2, 3, and 4, respectively. Tests

1 and 5 were with bulbs selected because of a high percentage of mild to moderate infection. In each case the bulbs were planted in non-infested soil. These tests covered two growing seasons (1944–45 and 1945–46) and gave results typical of those obtained by the majority of the growers who used Puratized N5E on the 1945–46 crop.

These results agree well with the laboratory and greenhouse data. A 1–4000 solution of Puratized N5X or N5E was not so effective in the field test as in many of the laboratory tests. In the field tests only enough solution was used to cover the bulbs. When a 1–4000 solution was used in this manner in the laboratory (Table 2) control was likewise poor. A 1–4000

TABLE 5.—*The effects of Puratized N5X and N5E on the development of black scale on previously diseased bulbs treated and planted in clean soil in the field*

Test No. and Material	Concentration	Time (Hours)	Number of bulbs harvested	Number of clean bulbs	Number of diseased bulbs in each class[b]			Disease index[c]
					1	2	3	
1 Puratized N5X	1–1000	48	98	96	2	0	0	1
	1–4000	48	96	42	17	10	27	41
	1–1000[a]	6	36	27	3	1	5	19
Check	.....	.....	91	17	4	7	63	74
2 Puratized N5X	1–2000	48	300	258	42	0	0	4
Check	.....	.....	318	58	240	18	2	31
3 Puratized N5E	1–2000	48	600	562	13	16	9	4
Check	.....	.....	736	442	94	84	106	26
4 Puratized N5X	1–1000	24	300	272	27	1	0	3
	1–2000	24	300	277	12	9	2	4
	1–4000	24	300	216	53	9	22	15
5 Puratized N5E	1–1000	24	100	91	5	3	1	5
	1–2000	24	93	85	6	2	0	4
	1–4000	24	98	32	40	15	11	35
Check	.....	.....	94	20	12	12	50	66

[a] Reduced pressure for 15 min.

[b] 1 = Mildly diseased; 2 = moderately diseased; 3 = severely diseased.

[c] Clean, mild, moderately and severely diseased bulbs were given numerical values of 0, 33.3, 66.6 and 100 respectively. The disease index for any given treatment was obtained by summing the products of the number of bulbs in each class and the numerical value of the classes and dividing by the total number of bulbs.

solution is therefore inadequate for practical use. There seems to be little choice between a 1–1000 solution or a 1–2000 solution except that a 1–2000 concentration may be nearing the point of non-effectiveness. A treatment time in excess of 24 hours was of little value in field tests conducted on as nearly a practical scale as was possible.

SUMMARY

Several fungicides were tested for control of black scale of Easter lilies. Only one, phenyl mercuric triethanol-ammonium-lactate, known under the code and trade names of Puratized N5X, Puratized N5E, or Puratized Agricultural Spray, was effective in eliminating *Colletotrichum lilii* from in-

fected lily-bulb tissues in laboratory tests without severe injury to the host tissue. At concentrations of 1–1000 to 1–2000 of the 10 per cent preparation, and with treatment for 24 hr., this fungicide gave practical control of bulb-borne infection in the field in two years, 1944–45 and 1945–46.

The eradicant action of Puratized N5E was inhibited by fungicides derived from thiocarbamic acid.

DEPARTMENT OF PLANT PATHOLOGY,
LOUISIANA AGRICULTURAL EXPERIMENT STATION,
BATON ROUGE, LOUISIANA.

A SEED–BORNE VIRUS OF MUSKMELON

WM. E. RADER,[1] HUGH F. FITZPATRICK,[2] AND E. M. HILDEBRAND[3]

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A mosaic disease of muskmelon caused by a seed-borne virus has been observed in New York plantings since 1936. In attempting to establish the identity of the causal virus it was found that transmission by mechanical means was readily achieved from melon to melon but not from melon to Turkish tobacco. Because of this it was suspected that the mosaic was not caused by the cucumber-mosaic virus (*Marmor cucumeris* var. *vulgare* Holmes). In 1941, experiments comparing an authentic cucumber-mosaic virus 1, a mild strain of cucumber-mosaic virus from Indiana, and the melon-mosaic virus on melon, cucumber, and tobacco made it apparent that the melon virus present in New York was unlike the cucumber virus.

The seed-borne muskmelon mosaic virus disease does not cause serious losses in New York judging by its known distribution and its relatively mild to moderate effects on the plants. The relationship of the virus under discussion to those on muskmelons in California and Michigan are not known.

The first report on a seed-borne virosis of muskmelons was made by Kendrick (4) in California who states that out of a total of 1,519 plants grown in the greenhouse from 23 packets of seed, only 27, or 0.23 per cent showed leaf symptoms within two weeks from planting. One hundred per cent infection was obtained by him in artificial inoculations on several varieties of muskmelon and summer squash, and symptoms comparable to those on Honey Dew muskmelon were obtained in 10 to 21 days after inoculation. The symptoms usually were severe, the leaves becoming reduced in size and modified in shape.

Mahoney (5) observed a seed-borne mosaic virus in inbred lines of muskmelon in Michigan. Infected plants showed typical mosaic on the first true leaf; their growth was slow in the field and setting of the fruit was delayed from 10 to 14 days. The mosaic was observed to spread rapidly through the field, so that by September 14 nearly all the plants were diseased. An average infection of 24 per cent was found in 6 out of 48 inbred progenies which showed mosaic. Further selections of these progenies always showed typical mosaic symptoms.

Middleton (6) describes a squash-mosaic virus disease which is seed transmitted. According to Freitag (in correspondence) this is the same

[1] Plant Pathologist, Shell Agricultural Laboratory, Modesto, California. Formerly assistant, Department of Plant Pathology, Cornell University, Ithaca, New York.

[2] Plant Pathologist, Food Machinery Corporation, Riverside, California. Formerly assistant, Department of Plant Pathology, Cornell University, Ithaca, New York.

[3] Professor of Plant Pathology, Texas A. and M. College, College Station, Texas. Formerly Assistant Professor, Department of Plant Pathology, Cornell University, Ithaca, New York.

virus which Kendrick (4) studied in muskmelons. Freitag believes that the disease is caused by a different virus from that encountered by Mahoney (5) because of the much higher percentage of seed transmission that Mahoney found.

Fields (1) records a squash-mosaic virus (not *Marmor cucumeris* var. *vulgare* H.) from California. He states (on the authority of Drs. H. H. P. Severin and J. H. Freitag) that the mosaic produced by this virus is the second prevalent squash disease in California.

SYMPTOMATOLOGY

In New York, symptoms appear on the first leaves of seedling muskmelons about two weeks after planting in the greenhouse (Fig. 1). Several

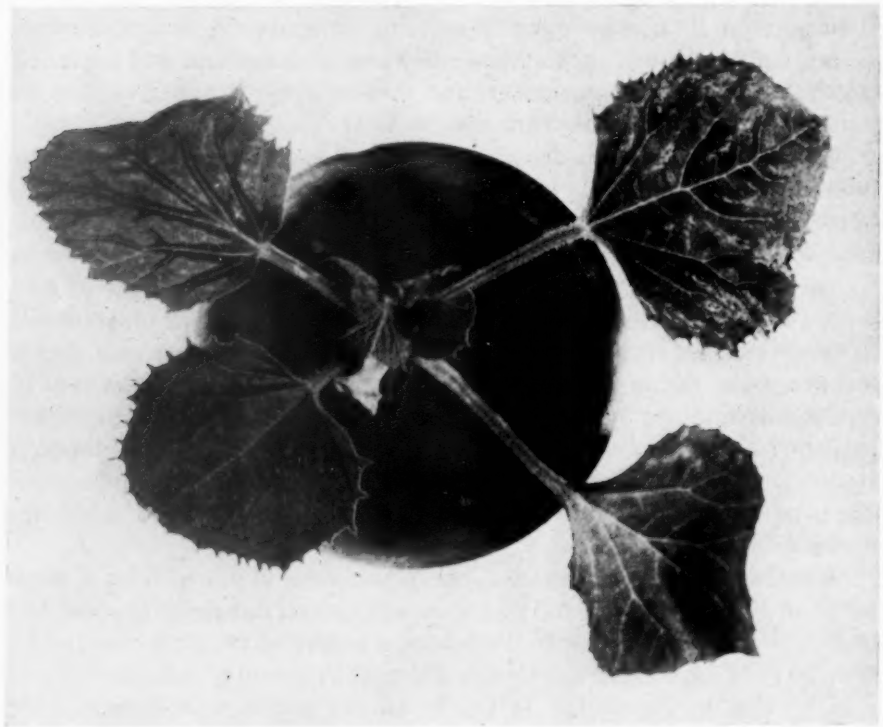

FIG. 1. New York muskmelon mosaic. Fourteen-day-old diseased muskmelon seedling, variety Queen of Colorado, showing veinbanding, distortion, and characteristic serration of the leaves.

different leaf symptoms may appear on the same plant (Fig. 2). The first leaf to show symptoms usually has a conspicuous veinbanding consisting of a narrow border of dark green parallel with each of the main veins of the leaf, the remainder of the leaf remaining the usual lighter green. The leaves which are formed later with rare exceptions do not show veinbanding, but are mottled without reference to the veins (Fig. 2, A). The leaves of the highly susceptible variety, Queen of Colorado, may, however, continue to

show veinbanding. The mottling varies from a coarse pattern of light and dark green through stages in which the leaves are stippled towards the edges with dark green, to cases where the mosaic symptoms are scarcely visible.

A peculiar serration is produced on some foliage in which the tips of

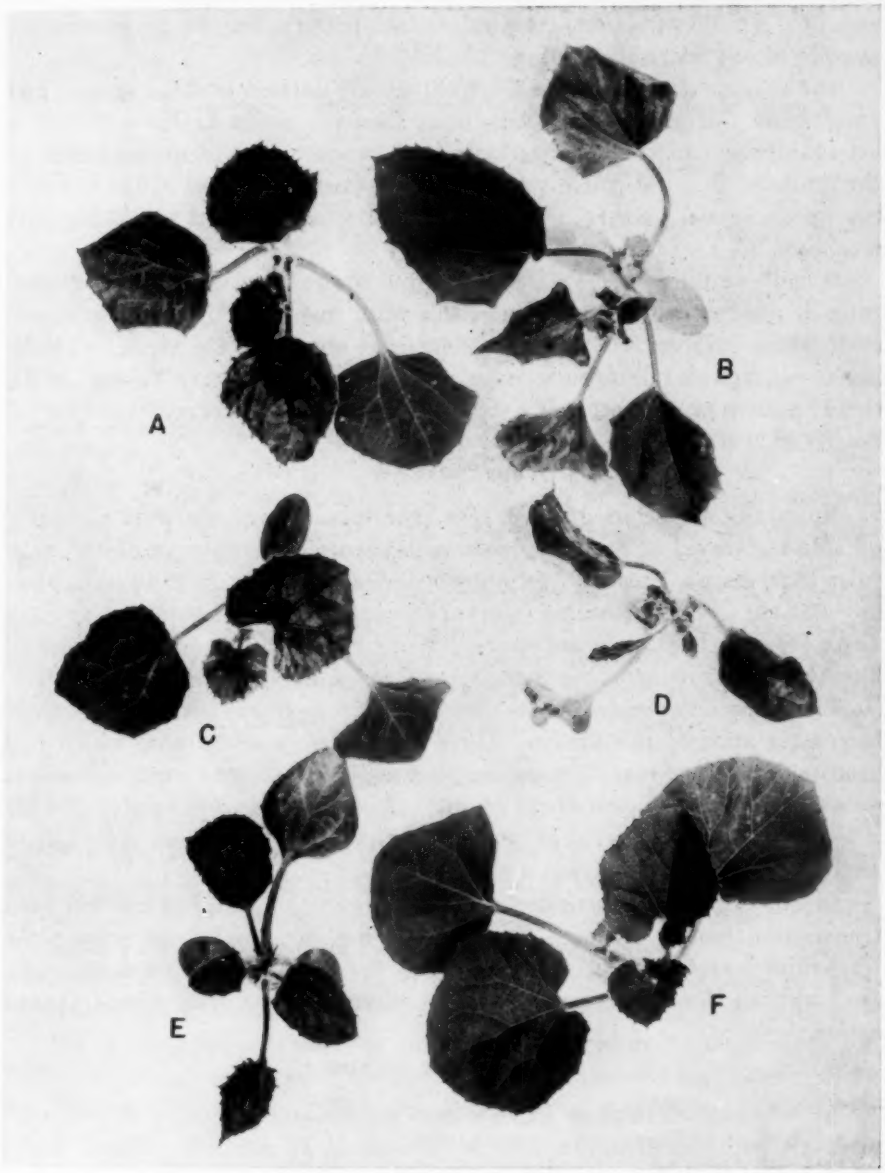

FIG. 2. Diseased muskmelon seedlings, variety Delicious, illustrating the range of symptoms of the New York muskmelon mosaic found on this very susceptible variety: A, seedling showing mottling and abnormal serration of the leaves; B, D, E, leaf distortions found in diseased seedlings; C, veinbanding of the young leaves; F, healthy seedling.

the veins protrude beyond the edge of the leaf, sometimes nearly one-eighth of an inch, giving a toothed appearance (Fig. 2, A and E). Abnormal serration may or may not accompany the mottling. Diseased leaves may also have deformations, which vary from an elongation and a curling in severe cases, to a rather smoothly outlined cordate pattern in mild cases (Fig. 2, B and D). In old infections the cordate leaf pattern may be present in the absence of any marked mottling.

The muskmelon virus gives a typical mosaic pattern in other species and varieties of Cucurbitaceae, although on *Cucumis sativa* L. the mottling is very faint and can easily be overlooked. There are no evident symptoms on the fruits of the susceptible varieties tested except on those of the summer crookneck squash, where they usually show as a green streaking and marbling.

Because of the extreme range of symptoms produced on inoculated seedlings, it was felt at the beginning of this work that there probably were several strains involved. Attempts to segregate strains of the virus by selecting one particular leaf pattern in serial inoculations have failed. Also, there has been no indication of a separation of strains in longevity or thermal inactivation studies.

TRANSMISSION

In studies on melon, 100 per cent transmission was obtained regularly by rubbing leaves of healthy plants with cheesecloth swabs saturated with juice from diseased plants. Symptoms appeared within 10 to 13 days after inoculation. This incubation interval corresponds to that obtained by Kendrick (4) with the California strain. The virus has been recovered from all parts of the plant including roots, stems, leaves, floral parts, and the pollen.

Transmission of mosaic virus through melon seed was observed regularly before the start of this study. Three lots of two-year-old seed which had been taken from three different diseased melons developed virus symptoms on 43, 24, and 12 per cent of the plants. Two lots of seed harvested in 1941 showed 93.5 and 27.9 per cent mosaic. Three years later these same lots of seed gave 2.6 and 6.4 per cent diseased plants.

In limited seed transmission tests in the greenhouse this virus has been transmitted readily through the seeds of the varieties of *Cucumis melo, Cucurbita moschata, C. flexuosus* and *C. pepo* (Summer crookneck). No seed transmission has been obtained in three separate tests with *Cucumis sativa.*

HOST RANGE STUDIES

All plants used in these experiments were inoculated twice at two-week intervals with carborundum dust being used as an abrasive. Three weeks after the second inoculation juice from these plants was inoculated into healthy muskmelon seedlings in an attempt to recover the virus. Table 1 lists the species of plants from which the muskmelon virus was recovered. All of these plants showed a mottling of the younger leaves with the excep-

tion of cucumber, in which the symptoms were either very faint or at times entirely masked.

TABLE 1.—*Species of plants from which the muskmelon virus was recovered five weeks after inoculation*

Family	Species and varieties[a]
Cucurbitaceae	*Cucurbita maxima* Duchesne (Hubbard, Buttercup, Golden Delicious) *Cucurbita moschata* Duchesne (Long Island Cheese) *Cucurbita pepo* L. (Summer Crookneck, Table Queen, Zuchini) *Cucumis anguira* L. (West India Gherkin) *Cucumis flexuosus* L. *Cucumis melo* L. var. *Chito* (Peach-vine or Mango melon) var. *conomon* var. *inordorus* (Persian melon) var. *reticulatus* (Bender, Coopers sweetheart, Davis perfect, Delicious, Honey Rock, Kilgores orange flesh rocky dew, Regular Iroquois, Smith perfect or yellow dew, and 13 × Honey Rock) *Cucumis sativa* L. (National pickle)

[a] Arranged according to the classification proposed by Tapley, Enzie and Van Eseltine (8).

A list of plants which showed no symptoms of the virus following inoculation and from which the virus could not be recovered by the method used in these tests is given in table 2.

Plants showing symptoms of the muskmelon mosaic described here have been readily inoculated with the cucumber 1 virus. The plants thus inoculated subsequently showed a stunting of the leaves and a rosetting of the terminal growths. The diseased progeny from the seed of these plants containing both viruses showed only the typical muskmelon mosaic.

CHARACTERISTICS OF THE VIRUS

The thermal inactivation temperature of the melon-mosaic virus, in expressed sap from diseased plants, is between 60° C. and 62° C. for a 10-minute exposure. Thermal inactivation studies were made by placing 3 cc. of sap expressed from diseased plants in thin-walled vials and lowering into a constant temperature water bath for 10-minute exposures over a temperature range of 54° C. to 74° C. Each test was replicated three times and three separate thermal inactivation tests were made; all gave identical results.

When attempts were made to inactivate the virus in the muskmelon seed with hot water treatments it was found that the thermal death point of the muskmelon seed was approximately the same as that of the virus. It is evident, then, that hot water treatments cannot be expected to destroy the virus within infected seed without killing the melon embryo.

In all tests the longevity *in vitro* has been more than 74 hours, with a maximum of 250 hours at laboratory temperatures. The sap from plants with mosaic symptoms was infective in all trials after having been dried on

TABLE 2.—*Species of plants from which the muskmelon virus could not be recovered five weeks after inoculation*

Family	Species
Apocynaceae	*Vinca rosea* L.
Chenopodiaceae	*Beta vulgaris* L. (Detroit dark red)
	Spinacea oleracea Mill.
Commelinaceae	*Zebrina pendula* Schnize.
Compositae	*Anthemis cotula* L.
	Aster sp.
	Calendula officinalis L.
	Chrysanthemum carinatum Schousb.
	Chrysanthemum segetum L.
	Helianthus annuus L.
	Lactuca sativa L.
	Rudbeckia hirta L.
	Zinnia elegans L.
Cruciferae	*Barbarea vulgaris* R. Br.
	Brassica arvensis (L.) Ktze.
	Brassica rapa L.
	Erysimum chieranthoides L.
	Raphanus sativa L.
	Roripa palustris (L.) Bess.
Polygonaceae	*Fagopyron esculentum* Gaertn.
Portulaceae	*Portulaca oleracea* L.
Scrophulariaceae	*Verbascum Thapsus* L.
	Verbascum blatteria L.
Solanaceae	*Browallia elata* L.
	Capsicum annuum L.
	Hyocyamus albus L.
	Hyocyamus niger L.
	Nicotiana rustica L.
	Nicotiana tabacum L.
	Petunia hybrida Viem.
	Solanum melongena L.
	Solanum tuberosum L.
Tropaeolaceae	*Tropaeolum majus* L.
Umbelliferae	*Apium graveolens* L.
Cucurbitaceae	*Citrulus vulgaris* L. (Dixie queen, Honey queen).
Gramineae	*Secale cereale* L.
	Triticum aestivum L.
	Zea mays L.
Labiatae	*Coleus blumei* Benth.
	Mentha spicata L.
Leguminosae	*Astragalus sp.*
	Baptisia sp.
	Glycine max Merr.
	Phaseolus lunatus L.
	Phaseolus vulgaris L. (Idaho refugee #5, Michelite).
	Trifolium pratense L.
	Vigna sinensis Endl.
Liliaceae	*Allium cepa* L.
Malvaceae	*Malva moschata* L.
Papaveraceae	*Eschscholzia californica* Cham.

glass slides for 72 hours, and a maximum of 298 hours was obtained in one test. The virus was no longer infective after three months in dried muskmelon leaves.

Sap from young leaves of mosaic plants remained infective in dilutions up to 1 : 2,500. No infection was obtained with dilutions of 1 : 3,000.

DISCUSSION AND CONCLUSIONS

The virus of muskmelons reported by Kendrick (4) is considered by Freitag (Middleton, 6) to be the same as the squash-mosaic virus. Presumably then, the thermal inactivation temperature of the melon strain must be the same (75° C.) as that reported by Freitag (2) for the squash-mosaic virus. The lower thermal inactivation temperature (62° C.) of the muskmelon-mosaic virus present in New York separates it from the squash-mosaic virus. The New York muskmelon-mosaic virus is distinguished from the common cucumber-mosaic virus by a more restricted suscept range and a much higher percentage of seed transmission.

The seed-transmitted melon-mosaic virus observed by Mahoney (5) in Michigan may be the same as the New York muskmelon-mosaic virus. However, in the absence of any specific data on the characters of the Michigan melon virus, and since the characters of the muskmelon virus found in New York differ from all other known viruses, the virus is described as new. The New York muskmelon-mosaic virus, as it is referred to locally, is characterized by a thermal inactivation point of the virus in expressed sap of 62° C. for a ten-minute exposure, by the veinbanding in the young leaves and the characteristic distortion and mottling in the leaves subsequently formed, and by the high percentage of seed transmission obtained in the progeny from diseased plants. Following the classification of Holmes (3) the name **Marmor melonis** sp. nov. is proposed for this virus.

The lethal virus of cantaloup occurring in the Imperial Valley of California (Middleton and Whitaker, 7) is presumably caused by a strain of cucumber-mosaic virus.

SUMMARY

A seed-borne mosaic virus of muskmelon, which has been present in New York plantings since 1936, is described as new. The disease caused by this new virus is characterized by a veinbanding and distortion of the young leaves, and a mottling of the leaves subsequently formed, by a thermal inactivation of the virus in expressed sap of 62° C. for a 10-minute exposure, and by the high percentage of seed transmission in progeny from diseased plants. The name *Marmor melonis* sp. nov. is proposed for the New York muskmelon-mosaic virus.

DEPARTMENT OF PLANT PATHOLOGY,
CORNELL UNIVERSITY,
ITHACA, NEW YORK.

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HOST SELECTIVITY AS A FACTOR IN THE ESTABLISHMENT OF PHYSIOLOGIC RACES OF TILLETIA CARIES AND T. FOETIDA PRODUCED BY HYBRIDIZATION[1]

C. S. Holton[2]

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INTRODUCTION

The appearance of previously unknown physiologic races of *Tilletia caries* (D. C.) Tul. and *T. foetida* (Wallr.) Liro at more or less frequent intervals is a primary factor contributing to the perpetuation of the wheat bunt problem. This is emphasized by the fact that races frequently attract attention first by their occurrence on presumably smut-resistant varieties. Consequently it has been suggested (7) that newly introduced wheat varieties actually act as "proving ground" for races of the bunt fungi that might otherwise remain obscure. The economic implications of this theory are so obvious that the problem deserves further consideration. In a recent paper (6) dealing with pathogenicity in hybrids of the bunt fungi it was suggested that the selective influence of the host played an important part in the expression of pathogenicity and therefore in the establishment of new races. This paper presents the results of a more complete study of this problem.

REVIEW OF LITERATURE

Several workers have demonstrated the effectiveness of host selectivity in separating specific races from mixed populations of races. According to Dillon-Weston (3, 4), certain resistant varieties were rendered completely susceptible by reinoculating them with bunt spores that came from these varieties. He attributed this to the selective influence of the host on the pathogen, which operates much like a plant breeder selecting biotypes from a population of mixed breeding material. Bressman (2) obtained similar results in his studies on physiologic specialization and concluded that new virulent races of bunt could be obtained by this method. Flor (5) separated one race each of *Tilletia caries* and *T. foetida* from a mixed population by the screening influence of several winter wheat varieties. On the other hand, a race of *T. caries* that had been propagated for several generations on its own differential variety was found to be pathogenically pure, as indicated by the lack of any screening influence on its virulence. Mourashkinsky (8) showed that the passage of a given strain of *T. caries* or *T. foetida* for

[1] Cooperative investigations of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, and the Washington State Agricultural Experiment Station, Pullman, Washington. Published with the approval of the Experiment Station Director as Scientific Paper No. 717.

[2] Pathologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.

five consecutive years through the same wheat variety considerably increased its virulence on that variety. He attributed this either to the screening influence of the varieties on a mixed population of races or to the production of a new race by mutation or hybridization. The latter explanation was regarded as the more probable and led Mourashkinsky (8) to the conclusion that, if such were the case, the problem of breeding wheats for bunt resistance was impossible of solution. Vielwerth (10) observed a decrease in pathogenicity of bunt populations constantly propagated on moderately susceptible wheat varieties whereas bunt populations from other varieties increased in pathogenicity when propagated on the moderately susceptible varieties. Bever (1) observed no increase in virulence on resistant varieties when pathogenically pure races were used while the pathogenicity of a hybrid population greatly increased with repeated passages through certain varieties.

For the most part these reports are concerned with results of studies in which spore populations of unknown pathogenic capabilities were used. In a study dealing with pathogenicity of hybrids between species and races of the bunt fungi (6) the writer obtained evidence that different pathogenic types were segregated and could be screened out and identified on an appropriate group of differential wheat varieties. Presentation of these results raised the question as to what selective influence, if any, would be exerted on hybrid bunt populations of this type by a completely susceptible host variety, such as Hybrid 128. Studies were undertaken to determine this point.

MATERIAL AND METHODS

Fifteen hybrids were selected for this study. This material was a portion of that used in an earlier study (6) on pathogenicity of hybrids. The wheat varieties used were Hybrid 128 (C.I. 4512), Albit (C.I. 8275), Hohenheimer (C.I. 11458), Hussar (C.I. 4843), and Hussar × Hohenheimer (C.I. 10068–1). To prevent infection by seed-borne spores from unknown sources, the seed was treated with a solution of formaldehyde (1 part to 320 parts water) for 10 minutes after which the formaldehyde was removed by washing the seed in running water.

Hybrid spores of the first generation were used to inoculate the susceptible variety Hybrid 128. Inoculum of the first segregating generation of the smut fungus (F_2) was taken from Hybrid 128 and in succeeding generations it was taken from Hybrid 128 and one or more of the other varieties. Inoculations were made by dusting the seed with spores, care being taken to prevent mixing the spores of different hybrids and different selections. The inoculated seed was planted in the field plots each fall and notes were taken the following summer. Smut percentages were based on counts of total and smutted heads to the row. The inoculations were continued for 3 years, or until the F_5 generation of the fungus was obtained, at which point the study was terminated.

EXPERIMENTAL RESULTS

It was shown in an earlier publication (6) that varietal selectivity is an important factor in the establishment of new pathogenic lines produced by hybridization. The results of this more recent study confirm that conclusion. In addition, however, these data reveal a distinct difference in the selective capacity of resistant and susceptible varieties (Table 1).

Tests were made with five hybrids between races T–8 and T–9. These races differ by the susceptibility of Albit to T–8 and its resistance to T–9 while Hohenheimer is susceptible to T–9 and resistant to T–8 (9). Hybrid 128 is susceptible and C.I. 10068–1 is resistant to both of these races. Hybrids between T–8 and T–9 were highly productive of new pathogenic entities. Some of these were more virulent and some less virulent than

TABLE 1.—*The percentages of smut produced in the F_3 generation by race hybrids of Tilletia caries, showing the selective influence of variety on the establishment of new pathogenic lines*

Smut races crossed and hybrid no.	Host variety on which inoculum was increased	Percentage smut on			
		Hybrid 128	Albit	Hohenheimer	Sel. 10068–1
T–8 × T–9					
50a	Hybrid 128	83	46	3	1
50b	Hohenheimer	84	10	57	2
50c	Sel. 10068–1	78	21	55	14
52a	Hybrid 128	85	13	1	0
52b	Sel. 10068–1	84	67	72	34
53a	Hybrid 128	64	2	6	1
54a	Hybrid 128	91	76	0	1
54b	Sel. 10068–1	92	9	60	23
55a	Hybrid 128	84	85	4	0
55b	Hohenheimer	86	73	61	4
55c	Sel. 10068–1	87	17	84	51
T–8 × T–10					
62a	Hybrid 128	93	51	77	4
62b	Hohenheimer	86	18	81	10
62c	Sel. 10068–1	90	70	89	63

either parent, depending upon the varietal source of the inoculum. The less virulent types (Nos. 52a and 53a) were established by repeated selection of the inoculum from Hybrid 128 while Selection 10068–1 established the more virulent types (Nos. 50c, 52b, 54b, 55c). Repeated selection of the inoculum from Hybrid 128 resulted in the recovery of the T–8 parent from three hybrids (Nos. 50a, 54a, and 55a) while the T–9 parent was recovered only when the inoculum was taken from Hohenheimer (No. 50b). New pathogenic entities virulent on C.I. 10068–1 were established only by repeated selection of the inoculum from that variety (Nos. 50c, 52b, 54b, and 55c). In addition to being pathogenic on this variety, all of these except 54b were virulent on both Albit and Hohenheimer.

One hybrid between races T–8 and T–10 was studied (Table 1). These races differ from each other in the same way as T–8 and T–9 (9), except

that T–10 is more virulent than T–9 on Hohenheimer. Repeated selection of inoculum of this hybrid from the susceptible variety Hybrid 128 established a pathogenic line that combines the attributes of both parents (No. 62a) whereas a line selected from Hohenheimer (62b) was only slightly different from the T–10 parent. A new, highly virulent type was established, however, by selection from C.I. 10068–1 (62c). Thus, the results obtained with this hybrid were similar to those obtained with the hybrids between T–8 and T–9, except that no lines less virulent than either parent were obtained. Less virulent types either were not produced by this hybrid or else they were missed by the random selection of inoculum from Hybrid 128.

Thus the selective influence of the host variety is a highly significant factor in establishing new races produced by hybridization. Furthermore, the nature of this selective influence appears to depend largely upon the degree of resistance of the varieties. Apparently the highly susceptible varieties, such as Hybrid 128, tend to promote the increase and establishment of segregates with low virulence. On the other hand, the highly resistant varieties, such as C.I. 10068–1, tend to promote the establishment of the more virulent types. This seems logical in view of the fact that only the highly virulent lines can infect the resistant varieties. However, there appears to be no reason why the highly susceptible variety should tend to select the less widely virulent types, unless these are produced in greater abundance than the more virulent types.

These results are substantiated in part by practical experience in the introduction of new bunt-resistant wheat varieties. As pointed out in an earlier paper (7) the establishment of a new, smut-resistant wheat variety usually is followed, sooner or later, by the appearance of a bunt race capable of infecting that variety. Consequently, in areas where new varieties are introduced, often the population of races is greater than in areas where long-established, highly susceptible varieties are grown (9). In other words, the results of this study, together with practical experience, emphasize again the need for a continued program of breeding resistant varieties and the development of more efficient seed treatment practices if bunt of wheat is to be effectively controlled.

CONCLUSIONS

1. Evidence is presented which shows that the selective influence of the host is an important factor in the establishment of physiologic races of the bunt fungi produced by hybridization.

2. The highly susceptible variety Hybrid 128 tends to promote the establishment of races with low virulence.

3. Highly resistant varieties tend to promote the establishment of races with high virulence.

4. These results are substantiated by practical experience. The population of highly virulent races is greatest in regions in which many new smut-resistant varieties are grown.

5. This study reemphasizes the importance of a continuous program of investigation on the bunt problem, embracing the genetics of bunt resistance, breeding bunt-resistant varieties, the biology of the bunt fungi, and seed treatment method and practices.

U. S. DEPARTMENT OF AGRICULTURE,
AGRICULTURAL EXPERIMENT STATION,
PULLMAN, WASHINGTON.

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VIRUS ATTENUATION AND THE SEPARATION OF STRAINS BY SPECIFIC HOSTS[1]

JAMES JOHNSON

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Some of the most obscure and important problems relating to viruses are associated with the origin and utilization of attenuated or mild strains of specific viruses. The protective or immunizing action of mild strains against more severe strains of the same virus is well known among virus diseases of both animals and plants. The causal agent of smallpox, for example, was known to be subject to attenuation by passage through cows or calves long before the virus character of disease was known. The true nature of such attenuation, transformation, modification, or alteration of a virus in either plant or animal hosts appears not to have been conclusively demonstrated. Similarly, little is definitely known about the origin of virus strains having greater virulence than the parent virus. In recent years there has been repeated evidence of the existence of mixtures of many virus strains in any ordinary virus culture. It has been generally accepted also that these strains, including attenuated strains, may arise through natural or induced mutation of the respective parent viruses. Such parent virus mixtures have been subdivided into other related group mixtures on the basis of symptomatology. The possibilities of isolating pure strains of viruses, originating for all practical purposes from single virus particles, offer new opportunities for securing more information on attenuation and mutation. The nature of the transformation of specific viruses from one degree of virulence to another on passage through suitable hosts is especially in need of investigation in order to promote more agreement in terminology (1, 17). The results with the ordinary tobacco-mosaic virus show that attenuation is due to the selective or differential response of a specific host to a mixture of severe and mild strains of this virus, permitting the separation of the latter from the former. According to the present results, the changes or mutations that created the mixture of strains occurred prior to the separation or attenuation by the host plant.

An abstract of this paper has been published (10).

SINGLE-LESION PURE-LINE STRAINS

Single-lesion strains often have been used in tobacco-mosaic-virus studies but little continued effort has been made to secure pure lines. During the course of the studies on attenuation, it became desirable to isolate and maintain single-lesion pure-line strains of tobacco-mosaic virus differing in their capacity to produce severe symptoms of disease. Until comparatively recently, a pure strain of a virus referred to a culture free from any other

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distinct or unrelated virus. Ordinary virus mixtures were easily separated by the use of differential hosts, properties, or vectors. However, it long has been known that different, though related, strains of a single virus frequently coexisted in ordinary virus cultures. Some workers believe that viruses are continually mutating and, on this basis, it would hardly be possible to maintain a culture that is not a mixture of strains (7, 15). This assumption is easily subject to experimental tests with the tobacco-mosaic virus and, in our experience, mutation is far less frequent than is generally supposed.

The single lesions produced on the hybrid host (*Nicotiana tabacum* × *N. glutinosa*), at virus dilutions as high as 1 to 10,000,000, are ideal for initiating pure-line cultures. The method is comparable to the Petri-dish culture method for isolating strains of bacteria and fungi. Hence, problems similar to those existing in Petri-dish isolations arise with virus isolation, and some improvements in methods need to be made and additional precautions taken in order to secure reliable virus isolations. The local-lesion host inoculated, for example, should be highly susceptible to infection and the virus applied at high dilutions together with frequent consecutive transfers from single lesions. The tobacco hybrid host is the most suitable yet found for this purpose. It is preferable to start with an aged, diluted, and buffered inoculum to lower the clumping of the virus particles. Gentle wiping with a cheesecloth pad on a carborundum-treated leaf, followed by rinsing with water, permits a possible selection of single-particle local lesions in the first transfer. The single lesions, removed with a small cork borer (5/16 in.), are thoroughly macerated and highly diluted (from 1:10,000 to 1:100,000) for transfers. Several consecutive transfers to the hybrid and repeated checking on the ordinary tobacco host is essential to determination. Proof of a pure strain cannot be finally conclusive in the absence of actual physical single-particle manipulation; hence, reasonably satisfactory proof must depend upon continued transfer through different host plants without evidence of further separation into other strains except such as may be attributed to natural or induced mutation.

Extensive trials with single-lesion tobacco-mosaic strains have resulted in a remarkable constancy in symptom expressions in the individual strains. Interesting though rare exceptions were noted, such as the occurrence of a trace of yellow-mosaic strain in a pure-line strain. Attempted pure-line isolation of this yellow-mosaic strain by the single-lesion method could not be accomplished, although the strain could be increased in relative concentration. It may be assumed that the occurrence of the yellow strain resulted from contaminating particles inseparable from other virus particles present in only extreme dilution in the original isolations. The conclusions of McKinney (15) and others as well as our own observations on the yellow strain suggest that it is more likely to be a recurring mutation.

The strains apparently occurring with greatest frequency in an ordinary field or laboratory virus mixture of ordinary tobacco-mosaic virus are

those which may be classified as the "severe" and "mild" strains, with several intermediary strains (Fig. 1). Our severe strain corresponds with strains described by others under the same name or other names indicating malformation, such as "distorting" strain. As yet it is not possible, on

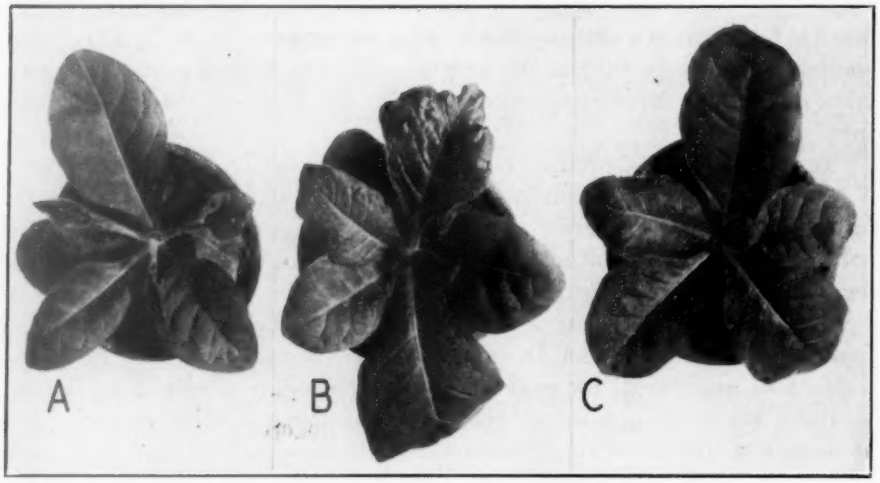

FIG. 1. Severe (A), medium (B), and mild (C) strains of virus on tobacco plants showing typical symptoms from single-lesion isolations. Photograph by Eugene H. Herrling.

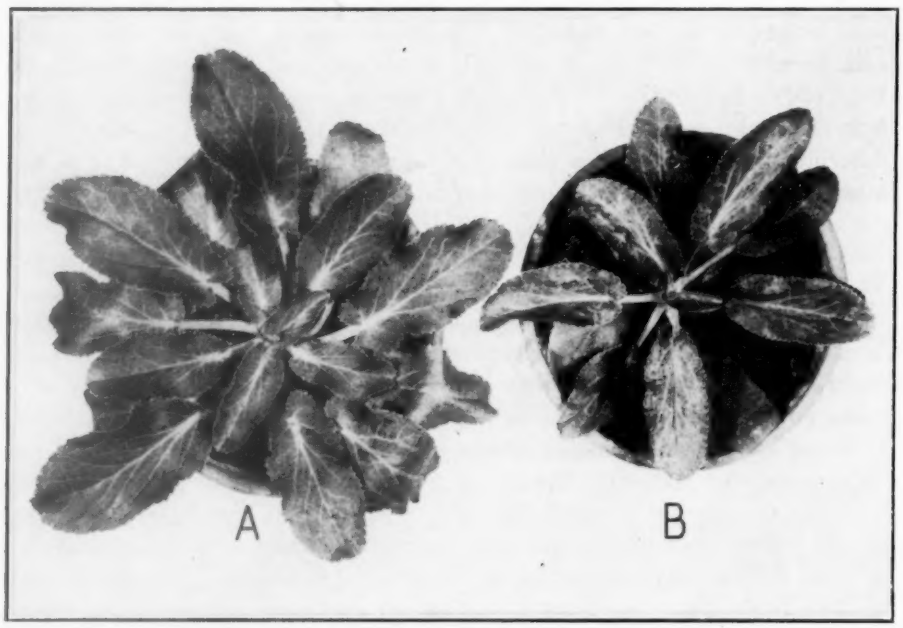

FIG. 2. The sea holly (*Eryngium*) host plant used in investigations on attenuation. A. Virus-free plant. B. Plant inoculated with tobacco-mosaic virus. No definite leaf symptoms result from this virus, except stunting, under some conditions, as shown here. Photograph by Eugene H. Herrling.

the basis of symptom expression alone, to state that one strain is completely identical with another arising from a different isolation; yet, unless one admits of no constancy in the nature of viruses, single-lesion pure-line strains may be grouped into a relatively small number of strains as far as reliable determination is concerned. Having obtained single-lesion pure-line strains of severe and attenuated viruses, it was possible to use and compare them on alternate hosts or to subject them to other treatments aimed at demonstrating attenuation or mutation.

THE NATURE OF ATTENUATION IN ERYNGIUM

In connection with efforts to unravel a puzzling mixture of viruses in the perennial sea holly or Buttonsnakeroot (*Eryngium aquaticum* L.), it was found that one of the viruses frequently present was that of ordinary tobacco mosaic (Fig. 2). The determination of the disease complex in sea holly was made more difficult by the fact that invariably the tobacco-mosaic virus component was present as a mild or attenuated strain. Laboratory inoculations consequently were made to sea holly with ordinary tobacco-mosaic virus, yielding severe symptoms. Infection occurred on the inoculated leaves and the original type of virus was recoverable from the inoculated symptomless leaves. The new, noninoculated and symptomless leaves of this slow-growing plant were repeatedly tested and eventually yielded virus. The virus recovered from these leaves was clearly not the same virus type as had been used for inoculation. Instead of yielding the severe symptoms of the parent virus on tobacco, the symptoms were always characteristic of a mild type. The virus originally yielding the severe disease on tobacco had been modified, transformed, or altered by the sea-holly host. If the parent virus is assumed to have mutated, all of the virus originally introduced must have undergone change, a supposition quite out of line with the accepted meaning of mutation. Consequently, some more logical explanation was sought for this behavior. One obvious method of investigation was to inoculate virus-free sea-holly plants separately with single-lesion pure-line strains of severe and attenuated tobacco-mosaic virus. After first testing the plants to prove freedom from virus, one series was inoculated on lower marked leaves with only a mild virus strain, and a corresponding series with a severe virus strain. At about weekly intervals, the inoculated leaves and the new leaves were tested for virus by removing small leaf discs with a cork borer, crushing this tissue in a small amount of water (1 cc. per disc), and inoculating to the tobacco-hybrid test plant, yielding local lesions. In this manner the progress of infection could be followed in the host. Reinoculations from local lesions back to ordinary tobacco determined whether the mild or severe strain of the virus had been recovered. In many cases the results were simplified by the fact that the mild strain yielded definitely smaller lesions than did the severe strain on the same leaf of the hybrid (Fig. 3). The results of a series of tests are shown in table 1. The severe form multiplied slowly but did not move out

of the inoculated leaves into new leaves. Neither did the severe strain from this source yield any transformed single-lesion isolations. The mild form multiplied more rapidly in the inoculated leaves, moved into the new leaves, and remained as mild virus during the full year of these reisolations (Table 2). Some minor exceptions of undetermined significance

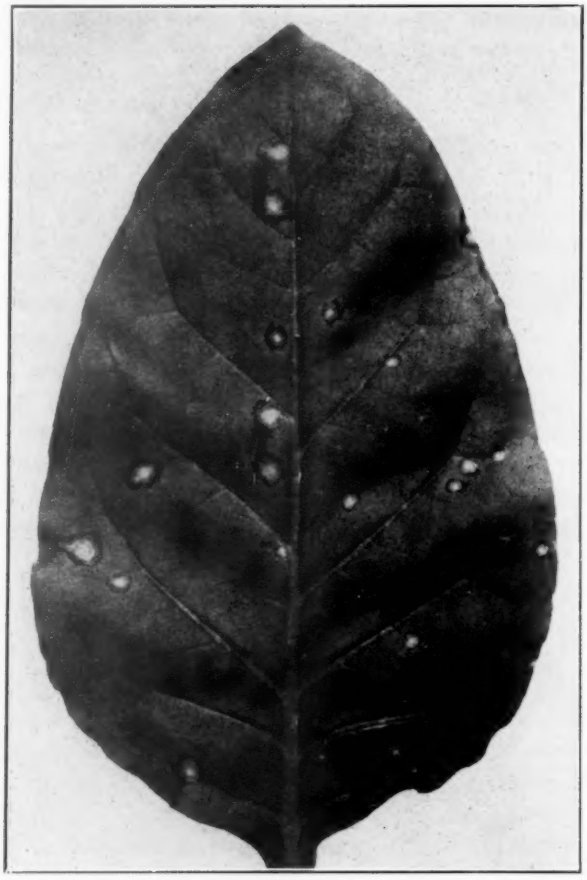

FIG. 3. A leaf of the tobacco hybrid host (*Nicotiana tabacum* × *N. glutinosa*). The left half of the leaf was wiped with a high dilution of the severe strain of tobacco mosaic and the right half with a mild (attenuated) strain. Note the smaller lesions on the right half (upper half in the illustration). Photograph by Eugene H. Herrling.

occurred during the later period of the tests. The sea-holly host is thus capable of separating (screening or filtering) two closely related strains of the same virus inoculated simultaneously to the plants. This separation is comparable to the longer known and more easily demonstrated separation of distinct and unrelated viruses by specific hosts.

Since, in the preliminary tests, an ordinary strain mixture of the tobacco-mosaic virus was inoculated to the sea holly and only a mild strain was recovered, the original virus was clearly attenuated according to the

accepted definition of this term since the work of Jenner (6) and Pasteur and his colleagues (16). The attenuation in *Eryngium* is thus shown to be only a matter of separation due to failure of the severe form of the virus to become systemic and to move into the new or noninoculated tissue. That other plants are capable of yielding similar responses with the tobacco-mosaic virus is probable, but the limited search for such plants has not been fruitful. Belladonna (*Atropa belladonna* L.), potato (*Solanum tuberosum* L.), digitalis (*Digitalis lanàta* Ehrh.), and tomato (*Lycopersicon esculentum* L.) were tested in some detail with negative results in this direction. Single-lesion isolations from inoculated infected leaves or from noninoculated leaves, if infected, yielded no attenuated virus, as illustrated by belladonna (Table 3).

TABLE 1.—*Summarized results of tests for virus in inoculated and noninoculated leaves of Eryngium made at intervals over a period of 7 months after inoculation of marked leaves with single-lesion pure-line severe and attenuated strains of the ordinary tobacco-mosaic virus*

Virus strain inoculum	Plant designation	Inoculated (lower) leaves			Noninoculated (new) leaves		
		Number of tests	Number yielding virus	Strain of virus recovered	Number of tests	Number yielding virus	Strain of virus recovered
Severe	A	4	2	Severe	11	0	.....
	B	4	3	Severe	5	0	.....
	C	3	2	Severe	7	0	.....
	D	3	1	Severe	6	0	.....
	Total A, B, C, D	14	8	Severe	29	0	.....
Attenuated (mild)	F	5	5	Mild	6	6	Mild
	G	3	3	Mild	5	5	Mild
	H	2	2	Mild	4	4	Mild
	I	2	2	Mild	5	5	Mild
	Total F, G, H, I	12	12	Mild	20	20	Mild

Further investigations on the sea holly have revealed that the severe strain of the virus in the inoculated and infected leaves eventually may become lower in concentration or disappear from these leaves. The older leaves, in any case, finally will die and be cast off the plant. Thus the host actually recovers from infections with the severe strain of the virus; and plants, once infected with the severe strain only, again may become susceptible to new infection with the same or other strains. The attenuated strain, on the other hand, persists in the host and protects it against the severe strain. The attenuated strain not only infects all the above-ground tissues, which are normally destroyed by freezing to the ground level during the winter, but permeates the crown and roots of the perennial sea holly and develops in the new spring growth. Some difficulty has been experienced in obtaining infection on some individual plants of *Eryngium*, indicating high resistance to both strains of the virus. Upon reinoculation (Table 2,

TABLE 2.—*Summarized results from reinoculations of new leaves of the same Eryngium plants as shown in table 1 with single-lesion pure-line strains of tobacco-mosaic virus. Tests for virus made at intervals 7 to 12 months after first inoculation*

Plant	Inoculation and strain of virus			Inoculated leaves			Noninoculated leaves		
	First April 11, 1946	Second Oct. 28, 1946	Third Dec. 7, 1946	Number of tests	Average number of lesions on 5 half-leaves of hybrid	Strain of virus recovered	Number of tests	Average number of lesions on 5 half-leaves of hybrid	Strain of virus recovered
A, B, C	Severe	Severe	None	20	340	Severe	18	3	Severe
D, E	Severe	Severe	Mild	9	453	Severe after first and second inoculation; mild after third	11	869	Mild
F, G, H, I	Mild	Severe	None	8	1222	Mild after first; severe after second	5	710	Mild
J	Mild	Severe	Mild	3	166	None after first and third; undetermined after second[a]	11	2	Mild

[a] A significant amount of infection was found only on Dec. 6; plant J was resistant to systemic invasion by both strains.

plants D, E, J) only one plant (J) proved resistant to systemic infection with the mild strain.

SEPARATION OF A SEVERE STRAIN ON TOMATO

The sea-holly host accounts for the natural separation and occurrence of mild strains of a virus as distinct from the more common and severe mix-

TABLE 3.—*Summarized results of tests for virus in young tomato and belladonna plants at intervals of 2 to 22 days following inoculation of lower marked leaves with severe and attenuated strains of the ordinary tobacco-mosaic virus*

Test plant and strain of virus inoculum	Number of plants inoculated	Inoculated leaves			Noninoculated leaves		
		Number of tests for virus	Average number of lesions on 5 half-leaves of hybrid	Strain of virus recovered	Number of tests for virus	Average number of lesions on 5 half-leaves of hybrid	Strain of virus recovered
Tomato							
Severe	5	5	840	Severe	7	1400	Severe
Attenuated (mild)	16	23	1.5	Mild	13	0.8	Mild
Belladonna							
Severe	8	8	786	Severe	30	1	Severe
Attenuated (mild)	8	8	370	Mild	24	3	Mild

tures. The reverse situation also has been suspected in the field and laboratory. Occasionally, severe strains of specific virus diseases (evidently obtaining no protective influence from mild strains infecting simultaneously) occur naturally, almost as though single-lesion laboratory selection had preceded. Such a selection by a host plant has been found to occur with the ordinary tobacco-mosaic virus on the tomato. When lower marked leaves of tomato (*Lycopersicon esculentum* L. var. Marglobe) are inoculated with virus of the ordinary severe tobacco mosaic, the severe mosaic virus is recoverable from both the inoculated and noninoculated leaves. When a single-lesion pure-line severe strain of the virus was used, the results were the same; however, when a single-lesion pure-line mild strain of the tobacco-mosaic virus was used for inoculum, the results were strikingly different. The virus could not be recovered from either the noninoculated or the inoculated leaves (Table 3). The situation was the reverse of that in *Eryngium* since the severe strain became systemic and differed also in the fact that the mild (attenuated) strain of the virus did not infect, the tomato plants used being naturally immune. The separation of the two strains, therefore, occurred at the point of inoculation rather than in the tissue itself. It cannot be said as yet that all tomato varieties are immune from any one attenuated strain or that one tomato variety is immune from all attenuated strains, although this seems likely. This behavior on tomato is highly indicative of the manner in which virus strains of high virulence may arise under natural conditions as contrasted to methods of laboratory isolation.

A limited number of other plants have been investigated for reactions similar to that of the tomato but none has been found. The considerable number of known solanaceous hosts (4, 5), as well as those outside this family, suggests other possibilities. Many of these are susceptible, either locally or systemically, without the development of symptoms. The testing of many species with different strains of viruses, when involving single-lesion reisolation to determine possible modifications of the virus itself including that in symptomless tissues, is a laborious and time consuming task. However, there are possibilities of developing new and short-cut methods, as is illustrated by the discovery of more reliable differentiation of the local lesions themselves, than is possible on the hybrid host normally used for that purpose in our tests.

SELECTIVE LOCAL-LESION REACTIONS BY ATTENUATED STRAINS

Wiping the leaves of the tobacco variety Havana 38 with either the attenuated or severe strains of tobacco-mosaic virus yields no inoculative local necrotic lesions or symptoms but only the typical systemic symptoms of mottling, stunting, or malformation. This type of response is characteristic of most of the common commercial varieties of *Nicotiana tabacum*. The hybrid *N. glutinosa* × *N. tabacum*, regularly used as the local-lesion test plant in our laboratory, yields only local lesions on the older and larger

plants used for this purpose. If young hybrid plants are used, however, the severe strain produces systemic necrosis, entering the veins, midrib, stem, and bud, and often killing the plant to the soil level. The attenuated strains, however, remain localized even in young plants, indicating less capacity for movement. When the age and condition of the hybrid host are favorable, both strains remain definitely localized, but the lesions of the severe strains may be larger than those of the attenuated strains, making it relatively simple to select and isolate strains of different degrees of severity from a mixed virus culture. When the single-lesion pure-line severe and attenuated strains are wiped on opposite sides of the same hybrid leaf,

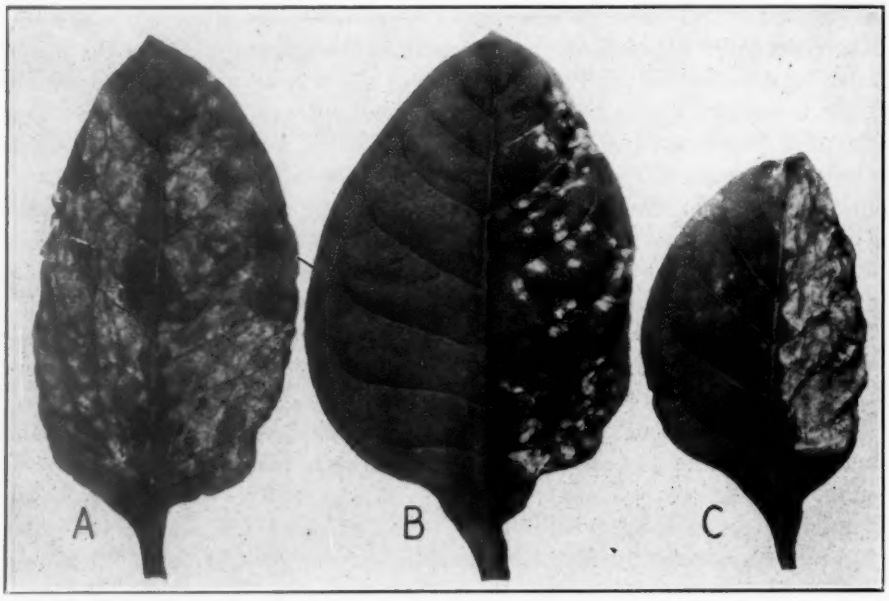

FIG. 4. The left side of each of the three leaves shown was inoculated with a severe tobacco virus strain and the right side with a mild (attenuated) strain of the same virus. A. The *tabacum-glutinosa* hybrid. B. Var. Daruma. C. A *tabacum-longiflora* hybrid. Note the different reactions for the severe strain of the virus on the left sides of the leaves. Photograph by Eugene H. Herrling.

the uniformity of the difference in size of the lesions is often striking (Fig. 3). Selection on the basis of size of lesion, however, is not a reliable criterion.

During a series of tests by the half-leaf wiping method, similar inoculations were made on a foreign variety of tobacco known as Daruma. Strangely enough, good necrotic local lesions developed on Daruma with the mild or attenuated strain but no local lesions developed from the severe strain on the opposite sides of the same leaves (Fig. 4). The severe strain infects Daruma systemically or produces only faint chlorotic areas on the inoculated leaf. The mild strain is also capable of systemic infection on young plants of Daruma. This unexpected response naturally suggested

similar tests on other varieties of *Nicotiana tabacum* and other species of *Nicotiana*. It was found that some species of *Nicotiana* responded in a similar manner to Daruma, *i.e.*, yielding necrotic lesions with only the mild strain. These species included chiefly *N. sylvestris* and *N. longiflora*. Other species of *Nicotiana* yielded local lesions with both strains (*N. glutinosa, N. rustica*) and others with neither strain, as is the case with most varieties of *N. tabacum*. Some other varieties of *N. tabacum*, like Daruma, yielded large or incipient local lesions with the mild strain (*e.g.*, Maryland Broadleaf, Xanthia, and Orinoco). The F_2 of a cross between *N. longiflora* and *N. tabacum* (Clayton's T.I. 106) yielded good local lesions with the mild strain only (Fig. 4). The parentage of Daruma is not known to us, but its appearance and virus response suggest that it possesses both *N. tabacum* genes and genes for local lesions with the mild strain of the tobacco-mosaic virus which originally was most likely derived from some other species than *N. tabacum*.

VIRUS MUTATIONS INDUCED BY HEAT

The yellow tobacco-mosaic virus apparently arises in nature as a mutant strain in the absence of any known contributing factor, unless it is more likely to develop on some hosts than on others. In a series of consecutive transfers of a single-lesion pure-line severe strain, through the hybrid and tobacco, one set of inoculations included 2 Havana and 1 Burley plant. A distinct yellow variant appeared on the Burley but not on the Havana plants, suggesting that the low-chlorophyll Burley had contributed to its origin, but no further evidence on this point was secured. We have observed, however, that some varieties of tobacco yield highly intensive symptoms of yellow mosaic as compared to others. McKinney (15) and Jensen (7) have concluded that the yellow mosaic forms arise occasionally as mutants rather than as contaminants. Earlier attempts made in this laboratory (11) to modify viruses themselves by passage through different hosts yielded only negative results except with Martinya, but single-lesion methods were not used at that time. The reported attenuation of the sugar-beet curly-top virus (2) in weed hosts has been questioned and apparently disproved (3, 14). As indicated above, we have sought for virus modification of pure-line severe strains by certain uncongenial hosts with only negative results. There appears to be no conclusive instance of mutation occurring in host tissue in which the virus multiplies except that reported for yellow tobacco mosaic virus.

The number and frequency of attenuated strains in field samples of tobacco mosaic suggest that some contributing conditions favor their occurrence. We have obtained mild strains from virus-infested soil and from aerated and fermenting extracts, indicating that extensive modification of virus particles may be concerned (9). In these earlier trials, single-lesion pure-line strains were not used, and it is quite as likely that some form of separation of severe and mild forms was occurring as that new strains were

formed. Attenuated strains (8) were obtained through the action of high temperatures (35°–37° C.) on virus infected plants but, again, the original inoculum was not pure-lined or known to be a strain free from mild strains.

Conclusive proof that a pure-line virus strain could be modified in any manner, therefore, remained to be secured. In view of our earlier trials, it seemed likely that this could be accomplished by allowing a single-lesion pure-line strain of severe tobacco virus to multiply at a constant temperature of about 36° C. Lower marked leaves or portions of leaves of several tobacco plants, consequently, were inoculated with a severe strain and incubated at 35°–37° C. in an artificially lighted air-control chamber for plant culture. Sample discs of both inoculated and noninoculated leaves were taken at intervals with a small cork borer and the discs either stored for future use on filter paper in covered Petri dishes or inoculated at once at a suitable dilution to the hybrid host which yielded local lesions. These local lesions were then selected according to size or appearance and inoculated to Havana 38 tobacco in comparison with the same strains that had not been incubated to determine the modifications, if any, that had resulted from the heat exposures.

The results showed conclusively that distinct modification developed in some particles of the pure-line virus during the incubation at 35°–37° C. The most frequent variants were in the direction of strains of reduced virulence (attenuation), although this may have been due in part to more frequent selection of small lesions for transfer (Table 4). Many of the

TABLE 4.—*The type and number of single-lesion strains isolated from Havana tobacco plants grown at 35°–37° C. after being inoculated with a single-lesion pure-line severe strain of tobacco mosaic. The periods of temperature treatment varied from 5 to 13 days, and the lesions isolated on the hybrid host were usually selected for relative size. Recovered strains were determined on the Havana variety*

Exp.	Number of plants at 35°–37° C.	Number of plants yielding attenuated virus	Number of small local lesions yielding no infection	Strains and number of times isolated from single lesions			
				Very mild	Mild	Severe	Undescribed or new
1	6	6	5	6	18	16	1
2	8	6	6	8	6	23	5

local lesions were very small and some, even when combined to yield more inoculum, failed to yield any infection whatever on transfer to tobacco. This suggested the development of strains capable of causing weak infections on the hybrid but too weak to be transferred further. Other lesions yielded no symptoms on tobacco but systemic infection was present; that is, the virus was latent in tobacco as could be demonstrated by inoculation back to the hybrid host where it yielded abundant local lesions. Other lesions yielded varying degrees of attenuation which could be perpetuated as

single-lesion pure-line strains in successive transfers. The majority of the lesions isolated from noninoculated leaves in the temperature-control chamber were evidently of the original severe strain, as judged by the size of the local lesion obtained, only about 5 per cent showing evidence of being transformed to the mild strain. The results in 2 series of temperature exposures were not the same in all plants tested, modification being greater in some plants than in others. Since only relatively small areas of treated plants could be sampled, and the sample discs were purposely selected from different leaf-positions and parts of leaves, much variation in this respect was to be expected. More detailed work would need to be done to trace the rate and type of changes developing.

The most surprising result of the heat treatment of the virus was the appearance of entirely new strains, not easily recognized as tobacco-mosaic

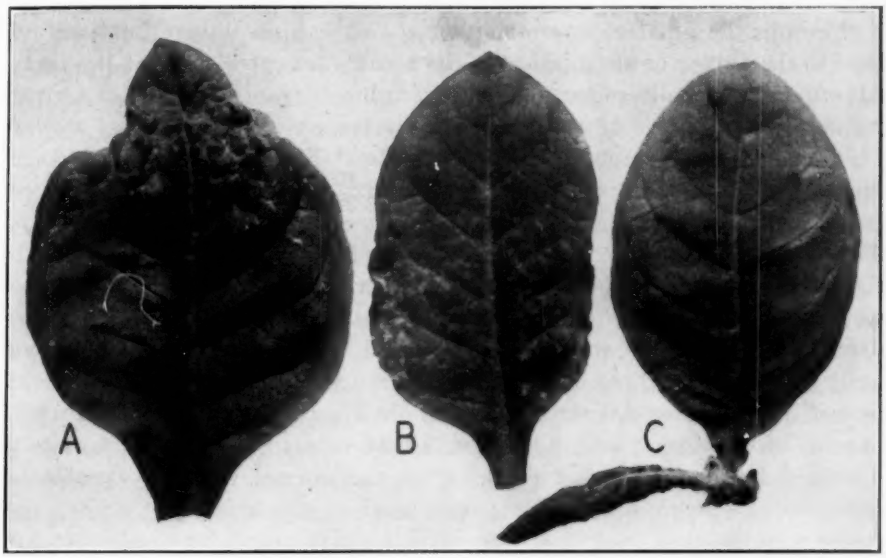

FIG. 5. Tobacco leaves infected by three single-lesion strains (A, B, C) of modified or mutated virus, originating from a single-lesion pure-line strain of severe tobacco-mosaic virus when grown for several days at 35°–37° C. These new strains, showing little or no resemblance to the parent virus, remained constant in continued successive transfers. Note especially the bud-infection symptom on C. Photograph by Eugene H. Herrling.

virus, some being more severe in certain respects on ordinary tobacco than the original severe malforming strain used for inoculum. Proof of their identity as single-lesion strains of the tobacco-mosaic virus was verified by the production of ordinary appearing local lesions of tobacco virus on the hybrid and *Nicotiana glutinosa* hosts and by thermal death-point and virus-aging determinations. One of these strains was referred to as the "bud-infecting" strain for the reason that only the bud of young plants became very chlorotic and usually was killed (Fig. 5). New, secondary buds were then formed, developing scraggly plants with malformed leaves but with little further evidence of continued necrosis or chlorosis on the older plants.

Other new strains were milder and the virus less concentrated, but they seemed to have some similarities to the bud-infecting strain (Fig. 5).

With the heat treatment, therefore, the origin by mutation of new strains of a virus from a single-lesion pure-line strain is believed to be demonstrated. No doubt similar mutations might be procured from single-lesion pure-lines by other methods as, for example, by chemical and X-ray or ultraviolet ray treatments. When single-lesion pure-line strains are not used, proof would need to be established that the transformation is not due to some form of separation of mixed strains as a consequence of differential sensitivity to the treatment. On the basis of our experience with single-lesion pure-line strains, there is little reason to doubt that some of these may be said to have come from a single virus particle.

DISCUSSION

Perhaps the greatest interest attached to the present investigations relates to the nature of attenuation in the broadly accepted sense of the term. Attenuation normally refers to the modification or transformation of a virus yielding severe types of symptoms or disease on its normal host to one yielding only mild symptoms on the same host. The attenuated virus, when inoculated to the host prior to or at the time of infection with the severe strain, normally protects the host from severe attacks of the severe strain. Among animal viruses, this principle has found wide application. Although attenuation has been obtained in other ways, the first and most common method is by passage of the virus to be attenuated through an alternate host or hosts that will yield the desired result. The virus must obviously be modified 100 per cent; otherwise the use of protective strains might be ineffective or even dangerous with certain diseases. It has been assumed, in some cases at least, that the alternate host causes the virus to "mutate," a term defined as a sudden change from the normal which is capable of continued perpetuation. A form of mutation, which is complete for the entire population of individual organisms or virus particles concerned, has not been demonstrated and is most unlikely. The use of such terms as "modification," "transformation," "alteration," or even "attenuation" has not implied anything specific as to the origin of mild strains of viruses. Since the early work on smallpox by Jenner (6) and on rabies by Pasteur (16), the nature of attenuation seems to have remained in some obscurity. There is ample proof of the existence of attenuated virus strains in plants, and their immunizing action against related, more severe strains of virus is closely comparable to the behavior of the animal virus strains. Since the term "attenuation" has been adopted for plant viruses from its usage in the animal virus field, additional reasons exist for comparing and correlating results in the two branches of research.

Attenuation of the ordinary tobacco-mosaic virus as it exists in nature by *Eryngium* (sea holly) is a result of the separation, screening, or filtering out of mild or attenuated strains from the more severe strains. Only the

mild strain is normally recoverable from the noninoculated tissues of the plant. The mutation of virus particles from which the attenuated strain is derived, must have occurred previously, so that the two strains existed as a mixture some time prior to infection and passage through the attenuating host.

Further support of this conclusion is to be found in evidence presented that related virus strains in mixture may be separated on the tomato plant on the basis of selective susceptibility to infection. In this instance the separation is reversed, the mild strains are eliminated, the severe strains are isolated and their occurrence in nature among milder strains thus is explained. Such natural occurrences of mild and virulent strains in a plant population are very suggestive of a similar situation in animal viruses.

The reversal of the behavior of related virus strains on genetically related hosts indicates a variety of new problems in need of further study, especially with respect to the inheritance of resistance in different strains of plants to different strains of the same virus. The production of new strains of viruses from pure-line strains by the simple process of the heat treatment of the multiplying virus also opens new avenues for basic research on mutation in viruses.

The separation of the virus strains on sea holly and on tomato are sharp and complete reactions. Separation of virus strains may be accomplished occasionally by hosts in which the rate of progress of infection into new tissues differs less strikingly. Such an instance was essentially accomplished in this laboratory by Koch (12) in dealing with the potato mottle and the potato ring-spot strains. In this and similar cases the purity of the isolations is less distinct and certain, even after repeated passage through the same host, and the alteration may be only one of relative concentration. Many viruses have been changed in virulence or in symptom expression by selections of this sort, but complete freedom of one strain from another is not assured unless the host is naturally highly selective or may be made more selective under special conditions such as a constant change in environment. In view of the local-lesion reaction of attenuated strains on *Nicotiana sylvestris* in our experiments, it would be interesting to repeat some of Kunkel's (13) experiments on acquired immunity in which he used *Nicotiana sylvestris* as a host plant.

SUMMARY

The true nature of attenuation as applied to plant and animal viruses has not been conclusively determined. In a similar manner, the natural origin of apparently new or highly virulent strains of viruses from normal parent viruses has remained obscure.

The well known ordinary tobacco-mosaic virus offers exceptional possibilities for investigating such phenomena. Through the use of proper methods and precautions, single-lesion pure-line strains may be isolated that, for all practical purposes, arise from single virus particles. The

pure-line strains remain remarkably constant, although it cannot be said that occasionally alterations do not occur as a consequence of mutation rather than as a result of contamination. Pure-line strains are essential to reliable studies on the nature of attenuation and mutation in viruses.

Naturally infected sea-holly (*Eryngium*) plants regularly yielded an attenuated strain of the tobacco-mosaic virus. Laboratory plants inoculated with an ordinary field culture of severe tobacco-mosaic virus yielded only an attenuated strain in the noninoculated or new leaves, although the severe virus infected the inoculated leaves as well. *Eryngium* plants inoculated with pure-line strains of severe and attenuated virus separately showed that the severe strain remained localized in the inoculated leaves and the attenuated strain became systemic. Consequently, the attenuation of the ordinary tobacco-mosaic virus is due to the ability of *Eryngium* to separate an attenuated strain from a severe strain when the two strains exist in combination.

In a similar manner it has been shown that the tomato is capable of performing the reverse separation; namely, of isolating a severe strain of the tobacco-mosaic virus from an attenuated strain of the same virus present in a mixture. Such selective capacity demonstrates a means by which more virulent strains may arise under natural conditions as contrasted to laboratory isolation. The loss of the immunizing influence of the attenuated strains also adds to the virulence of the separated severe strain.

The attenuated strains yielded good local-necrotic lesions on certain species and varieties of *Nicotiana*, whereas the severe strain did not produce lesions on these same hosts. This behavior offers an exceptionally good method for determining the presence of either the attenuated strain or the severe strain or both in any sample extract, especially when combined with inoculation to the ordinary hybrid host.

It has been shown that single-lesion pure-line strains of the tobacco-mosaic virus, normally remaining constant, may be induced to mutate at a relatively high rate by allowing virus multiplication to occur at temperatures of 35°–37° C. Both attenuated and new strains have been isolated in this manner.

UNIVERSITY OF WISCONSIN,
MADISON, WISCONSIN.

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THE RELATION OF MEADOW NEMATODES TO BROWN ROOT-ROT OF TOBACCO[1]

W. D. Valleau and E. M. Johnson

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For many years a root-rot of tobacco that differs from black root-rot has been under observation in Kentucky. The dead roots are brown, or sometimes pink or purple, instead of black, and the cortex is usually reduced to a thin sheath loosely surrounding the stele. Growth of tobacco is very slow following transplanting. The leaves may wilt in the heat of the day. Roots of the original root system usually die early. Later, toward the middle of summer, new roots develop on the buried stems and plant growth is fairly normal if the soil is fertile. Some years there is a marked difference in wilting of tobacco following grass and following tobacco, the latter remaining turgid on hot days while tobacco following orchard grass, for instance, wilts severely. Other years there seems to be little, if any, difference. That the trouble is not caused alone by turning under sod is indicated by the fact that tobacco following an old bluegrass sod, on the Station farm, that probably never before had been plowed, grows rapidly, while tobacco following a good bluegrass sod in an old cultivated field may develop severe root-rot injury. The disease as it occurs in Kentucky has been called brown root-rot because it was believed to be similar to brown root-rot as it occurs in Connecticut, Maryland, Wisconsin, and Ontario.

At the Tobacco Disease Council meeting held at Tifton, Georgia, in the summer of 1946, meadow nematodes were discussed as a cause of injury to tobacco roots, by Jenkins and Graham. Technics for examination of roots for meadow nematodes and their identification were considered, and some discussion resulted as to the possible relation of meadow nematodes to brown root-rot. That meadow nematodes have been almost completely neglected in this country as a cause of root disease was brought out by Steiner in 1945.[2] In the spring of 1945 Doctor Steiner, in conversation, suggested that meadow nematodes might be the cause of brown root-rot in tobacco.

Following the Tifton meeting an examination was made of roots of tobacco plants from the Station brown root-rot plots at Lexington. Roots of several Burley varieties of tobacco were infested with what appeared to be the meadow nematode, *Pratylenchus pratensis*. Examinations were then made of tobacco roots from 132 other plots in the tobacco rotation and fertilizer series in all of which tobacco has started slowly in past years. Meadow nematodes were found in every sample collected.

Washed roots were kept overnight in a Petri dish of water. In the morning the roots were removed from the dish and the nematodes picked

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

[2] Steiner, G. Meadow nematodes as the cause of root destruction. Phytopath. 35: 935–937. 1945.

from the bottom of the dish with the aid of a stereoscopic microscope. At first the nematodes were removed with a bamboo pick, but later much more efficiently with a capillary pipette into which a nematode was drawn very quickly if the tip of the pipette was placed near it. Final examination was made with a compound microscope. Nematodes with a spear in the mouth parts were considered to belong to the meadow nematode group. It was not unusual to find several hundred meadow nematodes in the bottom of a dish in which a collection of tobacco roots had been left overnight.

Examinations were also made by placing small rootlets on a microscope slide in a small amount of water and, under a microscope, gently scraping the small brown lesions with a small scalpel until the nematodes emerged. Many meadow nematodes were removed from small brown lesions on the roots in this way.

These observations demonstrated that in the brown root-rot plots and in other plots in the rotation series where tobacco had started slowly, meadow nematodes were present in large numbers in washed tobacco roots, where they had apparently caused small brown lesions in the cortex. There is no reason, therefore, to believe that brown root-rot as it occurs in Kentucky is different from what is called meadow nematode root-rot in the flue-cured tobacco areas of Georgia, the Carolinas, and Virginia.

There is also reason to believe that brown root-rot as it occurs in Ontario, Canada is caused by the same agent that causes the disease in Kentucky. We have been studying the relative resistance of varieties of Burley tobacco to brown root-rot in the field for several years. The trials have included the Burley varieties Canada 354, susceptible; Canada 364, resistant; and Harrow Velvet, highly susceptible in Ontario to brown root-rot. The tests also included Ky. 33 and a local Kentucky variety, Canadian; both are resistant to black root-rot and resistant to brown root-rot at Lexington, Kentucky. These varieties were sent to Ontario for trial in brown root-rot plots. The five Burley varieties, including two susceptible and three resistant ones, gave the same reactions to brown root-rot at the two locations, strongly suggesting that the cause of brown root-rot is the same in both areas.

From a study of the literature on brown root-rot as it occurs in Wisconsin, Maryland, and other northern states, it is probable that the meadow nematode is concerned in brown root-rot in those areas also.

GROWTH OF TOBACCO UNDER DIFFERENT SYSTEMS

In contrast with the slow growth of tobacco following sod crops on the rotation series is the rapid growth, following setting, of tobacco in an old bluegrass field that had not previously been plowed in the history of the Kentucky Experiment Station. This field had been lightly pastured and had a heavy bluegrass sod. A small area is plowed each year for tobacco. The tobacco starts quickly, grows rapidly, and produces about 2000 lb. an acre. Twenty-seven collections of tobacco roots from this field were made; 16 were free from, 8 had a total of 42, and 3 a total of 302 meadow nematodes.

These results suggested that the nematodes present were limited to small areas. In the black root-rot plot and in 2 other plots tobacco is grown every year, with a small grain or a grain and hairy vetch cover crop. Although the fields are heavily infested with *Thielaviopsis basicola,* varieties highly resistant to this organism start growth quickly following transplanting and grow rapidly throughout the summer. Seven collections of tobacco roots from these fields gave only two meadow nematodes, while four collections of tobacco roots from the nearby brown root-rot plot yielded 1894 meadow nematodes. Fifteen collections of vetch roots during late fall and winter from the continuous tobacco fields had a total of only 62 meadow nematodes, and 14 collections of barley roots from the same fields gave a total of 26. One collection of vetch from the brown root-rot area nearby had 357, another vetch collection from the brown root-rot area had 992, while a barley collection from the same plot gave 199 meadow nematodes.

The system of continuous culture to tobacco does not appear to be favorable to the meadow nematodes under Kentucky climatic conditions, while long grass rotations with tobacco only occasionally seem to be very favorable to them. Burley tobacco growers in increasing numbers are growing tobacco year after year on the same land with a cover crop of a small grain and hairy vetch turned under about 3 weeks before transplanting tobacco. This system has resulted generally in tobacco that starts growth quickly following transplanting, grows uniformly and rapidly throughout the summer, and shows no evidence, from the standpoint of growth, of brown root-rot injury. In contrast, fields following bluegrass sod, alfalfa, lespedeza, orchard grass, and other pasture mixtures frequently show distinct evidence of brown root-rot, and in the few cases studied meadow nematodes have been present in the roots.

DISTRIBUTION OF MEADOW NEMATODES

No general survey of meadow nematodes in Kentucky has been attempted, but in the course of the present studies enough evidence has been gained to show that they are by no means limited to the Experiment Station plots. They have been found in tobacco roots from Simpson, Fayette, Henry, Boone, Owsley, Taylor, Clark, Warren, and Montgomery counties; in the roots of grass and clover from Bourbon, Scott, Woodford, Fayette, and Boone counties; in the roots of *Nicotiana rustica* from Jefferson County; and in tobacco roots from Ohio County, Indiana. These results suggest a rather general distribution in pasture and forage crop roots.

MEADOW NEMATODES IN THE ROOTS OF OTHER CROP PLANTS

It has been pointed out repeatedly by others that brown root-rot is severe following certain grass crops. If meadow nematodes are the cause of brown root-rot, then one should expect to find the nematodes in the roots of plants that precede a brown-root-rot-affected crop of tobacco and not in the roots of plants preceding a healthy crop. Filipjev[3] and Stekhoven include species

[3] Filipjev, I. N., and J. H. S. Stekhoven, Jr. A manual of agricultural helminthology. I–XV, 878 pp. E. J. Brill, Leiden. 1941.

in 34 plant families in the host range of *Pratylenchus pratensis.* In the present studies we have examined the roots of most of the plant species growing in 118 of 338 plots, in the tobacco rotation series, not in tobacco in 1946 but to be planted to tobacco in 1947. Grass and legume crops on this series of plots have never been so luxuriant as would be expected, considering the fertility of the soil. Roots from one plot only were without meadow nematodes, indicating that practically the whole series of plots is infested.

Meadow nematodes apparently identical with those found in tobacco roots were present, often in large numbers, in the washed roots of orchard grass, crab grass, bluegrass, timothy, redtop, fescue, and corn; in red clover, lespedeza, alfalfa, sweet clover, and soybeans; and in the weeds, *Sida spinosa, Oxalis sp., Cyperus esculentus,* dandelion, *Plantago lanceolata,* dock, *Erigeron canadensis, Rumex acetocella,* knotweed, pigweed, *Solanum carolinense, Physalis sp.,* and in *Lactuca scariola,* in which they are unusually abundant. Ragweed roots from plots where other plant roots were heavily invaded yield only an occasional meadow nematode. According to Lunn and associates[4] tobacco makes excellent growth following a crop of ragweed as compared with that following other weeds and natural weed fallow. With heavy meadow nematode infestation of grass roots and roots of other perennials in the fall and winter, it would be expected that abundant invasion of newly developing tobacco roots would take place following transplanting because the roots of the tobacco plants are set directly in the upturned roots of the preceding crop.

DISCUSSION

The results of the present study have shown that meadow nematodes are abundant in the roots of tobacco plants affected by what we have called brown root-rot; and that the roots of crop plants that precede tobacco and are known to produce conditions favorable for the development of brown root-rot are also heavily invaded with meadow nematodes in an area where brown root-rot occurs every year in tobacco. In other fields on the Station farm where tobacco starts growth quickly following transplanting, meadow nematodes have either not been found or found in very small numbers. While final proof is not presented that brown root-rot is actually caused by meadow nematodes and by nothing else, at least there is good evidence that the nematodes cause extensive injury to the roots of tobacco and of several other crop plants, and that this injury is of the type usually described for brown root-rot. It is also evident that meadow nematodes must be given serious consideration as a cause of failure or partial failure of other crops, as the small grains, corn, grasses, and legumes in Kentucky and neighboring states.

KENTUCKY AGRICULTURAL EXPERIMENT STATION,
LEXINGTON, KENTUCKY.

[4] Lunn, W. M., D. E. Brown, J. E. McMurtrey, Jr., and W. W. Garner. Tobacco following bare and natural weed fallow and pure stands of certain weeds. Jour. Agr. Res. [U.S.] 59: 829–845. 1939.

REPORT AND ABSTRACTS OF THE TWENTY-NINTH ANNUAL MEETING OF THE PACIFIC DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The 29th annual meeting of the Pacific Division of the American Phytopathological Society was held in San Diego, California, June 17–19, in conjunction with the meetings of the Pacific Division of the American Association for the Advancement of Science. Approximately 60 persons were in attendance from Arizona, British Columbia, California, Nevada, Utah, Washington, and Territory of Hawaii. June 17 and 18 were devoted to presentation of papers, 2 short business meetings, and a symposium on "Fungus Root Diseases of Crop Plants," with an all-day field trip June 19. Twenty-three formal papers were presented in addition to 10 presented in the symposium. George W. Fischer asked to be relieved of his duties as secretary-treasurer and his resignation was accepted.

The following new officers were elected for 1948:

President: L. C. Cochran
Vice President: George W. Fischer
Secretary-Treasurer: George A. Zentmeyer

Nineteen abstracts were submitted:

Disinfecting power of propylene oxide and propylene chloride in relation to phytopathogenic bacteria and fungi. PETER A. ARK. Propylene oxide gas at a concentration of 0.3 cc./l. inactivated *Xanthomonas phaseoli, Pseudomonas medicaginis phaseolicola, Ascochyta pisi,* and *Fusarium solani* f. *cucurbitae* in Petri dishes in 45 minutes under vacuum, at 25° C., while it required 0.5 cc./l. to kill *Verticillium sp.* during the same period of time. *Agrobacterium tumefaciens* and *Corynebacterium michiganense* are killed only beginning at 0.5 cc./l. of propylene oxide with an exposure of 60 minutes. Concentration of 1 cc./l. for 10 minutes, was lethal to all test organisms mentioned above. Propylene chloride was found to be lethal with concentrations beginning at 0.4 cc./l., with a minimum exposure of 12 hours. Higher concentrations shorten the period of exposure somewhat. No difference in dosages was observed whether the tests were performed *in vacuo* or not. Club wheat seed loses about 50 per cent in germination when treated by propylene chloride, 1 cc./l., under vacuum for 6 hours. No loss in germination was observed at 4-hour exposure and 1 cc./l. also under vacuum.

Effect of crystalline streptomycin on phytopathogenic bacteria and fungi. PETER A. ARK. Streptomycin (crystalline base) has been found to be toxic to fourteen species of phytopathogenic bacteria, both Gram positive and Gram negative. *Agrobacterium tumefaciens* had a considerable resistance to it. *Actinomyces scabies* is very susceptible to streptomycin, but *Fusarium solani* f. *cucurbitae, Phytophthora capsici,* and *Ascochyta pisi* were not affected by it. Cucumber, tomato, barley, oat, and sunflower seeds soaked 24 hr. in a streptomycin solution, as strong as 200 units per ml. showed no detectable injury and grew normally when planted in soil in a greenhouse. Cucumber seeds artificially contaminated with *Pseudomonas lachrymans,* and treated for 20 min. with the streptomycin solution containing 100 units per ml. were freed of the pathogen. Potato and carrot slices soaked in the streptomycin overnight and inoculated with *Erwinia carotovora* failed to develop soft rot. Development of *Erw. carotovora* was stopped when potato and carrot slices were inoculated and treated with streptomycin after intervals of 20 to 120 min. The results strongly suggest the possibility of using streptomycin against seed-borne phytopathogenic bacteria.

Stemphylium leaf spot of China aster. KENNETH F. BAKER and LILY H. CLARK. An unreported disease of China aster caused localized serious losses in cutflower fields near Redondo Beach, California, in mid-summer, 1946. The growers had saved seed from infected fields. Brown, circular or irregular coalescent spots, 1–10 mm. across developed abundantly on leaves, calyces, petals, and sometimes stems. *Stemphylium botryosum* Wallr. *sensu* Wiltshire sporulated on the spots, and its pathogenicity was demonstrated. Infection occurs through stomata and requires high humidity for 2 days; the spores proliferate for 24 hr., rupturing their walls before forming germ tubes. Conspicuous spots develop in 5 days. The fungus is seed borne and, during epigeal germination, sporulates on the seed coats, infecting the young plants. The disease is restricted to fields watered by overhead sprinklers in low areas protected from the drying effect of wind. This limitation is explained by the prolonged spore germination period. The disease should be controlled by using ditch irrigation and avoiding such low, humid areas. The fungus is distinct from *Alternaria zinniae, A. tenuis,* and the Alternarias reported as

Macrosporium florigenum and *M. caudatum* on China aster elsewhere. *Stemphylium botryosum* has been reported on China aster seed in Denmark.

The incidence and development of lemon shell bark. E. C. CALAVAN. Two types of pre-lesion symptoms of shell bark have been found about midway through the bark of mature Eureka and Lisbon lemon trees. Brown necrotic spots impregnated with a hard gummous substance, from 0.5 mm. to 3 cm. long, commonly precede "slow" shell bark lesions. "Fast," rapidly spreading lesions, however, frequently originate over thin tangential plates of discolored phloem. Considerable gum appears to be within the cells of these plates. Streaks of discolored phloem extend vertically from a plate ahead of the growing lesion. New lesions generally form during a flush of growth, particularly from February to May, and crack several months later. The first lesions of shell bark usually appear on the portion of the trunk having the thickest bark, commonly the south or east side just above the bud union. Fungi are apparently of little importance in the incidence of shell bark, but may accelerate lesion development. Numerous species of fungi have been isolated from the margins of advancing lesions, but no fungi have been found in 700 pre-lesions. Shell bark probably is due either to a virus infection or to physiogenetic factors.

Powdery mildew on sugar beet. EUBANKS CARSNER. Powdery mildew on sugar beet was observed in a few fields in the Yakima Valley near Toppenish, Washington, in the summer of 1945 and more extensively in that same valley in the summer of 1946. In the second season a small infestation was also noted near Nyssa, Oregon. In some cases, infected beet leaves showed a color reaction but no evidence was observed of serious injury. The perfect stage of the fungus has not been discovered.

Studies on the natural spread of the peach-mosaic virus among apricots, almonds, and peaches. L. C. COCHRAN and GILBERT L. STOUT. Early in the investigations of the peach-mosaic disease in Southern California, apricots and almonds were recognized as naturally affected hosts. Surveys showed a high percentage of affected trees in apricot orchards adjoining mosaic affected peach and old peach orchard sites, with less or none where peach has not been present. In a 500-tree experimental orchard of alternating peach and apricot planted alongside an old highly infected apricot orchard near which all other peach was previously removed only two peach and no apricot trees became infected during ten growing seasons. Similar rare cases have developed in several grower's peach orchards under parallel conditions. In another peach orchard approximately one mile distant from the experimental block, in which mosaic trees were allowed to stand, surveys by control agencies showed an annual increase, totaling 37.8 per cent during ten years. If the few cases of mosaic occurring in peach planted near mosaic affected apricots and almonds represent natural spread from these hosts, the rate is extremely slow in contrast to spread from peach to peach. No converse data were obtained but the apparent correlation of high incidence in apricot and proximity to mosaic in peach indicates rapid spread from peach to apricot.

Wood pocket, a newly reported disease of lemons. H. S. FAWCETT and E. C. CALAVAN. Wood pocket (ligno—cortosis) was first noted in 1937 in one strain of Lisbon lemons at the Citrus Experiment Station, Riverside, California. A defect or break in the bark is accompanied by discolored wood underneath. Later discolored regions are seen in a tangential cut as an irregular pattern of gum-filled tissue, darkly dotted on a lighter colored surface. The lesions vary from an inch in length to large lesions extending several feet along one side of the larger branches. Few to many small lenticular yellow to ocherous spots, averaging 0.5 to 1.5 mm., in the cambium of the trunk or branches is the first interior symptom. Trees propagated in 1939 by buds from apparently healthy branches of diseased trees developed wood pocket in 6 years. These, when top-worked to various citrus in 1946, have transmitted the leaf symptoms to healthy scions of several varieties of lemon. Some leaves have a variegated chimera-like aspect, with broad bands in shades of green to yellow to white or lace-like reticulations involving a part or the entire blade. Seedlings from seeds of mature lemons from diseased trees showed some plants with these leaf symptoms. The occurrence of leaf symptoms in seedlings, coupled with the transmission into healthy scions, indicates the presence of a virus.

Multiple sex factors in Ustilago striiformis f. hordei. GEORGE W. FISCHER. The sex reaction was studied of 46 monosporidial cultures of *Ustilago striiformis* forma *hordei*, taken from 12 collections of the stripe smut on species of *Agropyron* and *Elymus*, mostly from the western states. Previous investigations of the forma *hordei*, based on cultures from a single collection, had revealed only 2 sex groups. In the present work, only 2 groups were noted within any one collection of smut, whether or not the monosporidial cultures came from the same or different chlamydospores. When all 46 cultures

were paired up in all possible combinations on water agar, however, it was discovered that 6 distinct sex or compatibility groups were represented.

Some studies of curly top of flax. N. J. GIDDINGS. Specimens of diseased flax received from the southern San Joaquin Valley during the spring of 1945 were found to be infected with the curly-top virus. Since that time 37 species and varieties of flax have been grown in the greenhouse and tested to learn if they showed any differences in reaction to the various strains of curly-top virus. A few of the tests are incomplete at this time, but all commercial varieties have been susceptible to infection by each of the virus strains used and to severe injury by the more virulent strains. Some varieties have shown less injury than others as a result of infection and it is possible that further tests will give more evidence of such differences. The least amount of injury was induced by virus strains 7, 2, and 4 while strains 5, 6, 9, and 3 were highly virulent and resulted in a high mortality among the plants of most varieties. *Linum lewisii* Pursh. secured from Theodore Payne, seedsman, appears to be highly resistant. *Linum perenne* and *L. flavum,* secured from Aggler and Musser Seed Company, also appear to be very resistant, while *L. grandiflorum rubrum* from the same source does not. The experiments thus far have not given any indication of flax species or varieties which would be of help in differentiating curly-top virus strains.

The development of Pierce's disease and its occurrence in rogued and nonrogued vineyard plots. WM. B. HEWITT. Though the occurrence of Pierce's disease varied in different districts of the San Joaquin Valley, the general development and spread of the disease increased very rapidly after 1934 and reached a peak about 1941. The annual incidence has since declined in similar proportions. The occurrence of Pierce's disease in a vineyard, a district, and even the entire valley followed three general patterns; 1, irregularly scattered over most of the area; 2, centered in small localized areas; and 3, concentrated in portions of vineyards adjacent to alfalfa or irrigated pastures. The systematic removal of diseased vines twice each season, once in the spring and again in the fall, in vineyard plots varying in size from 10 to 120 acres each, did not significantly influence the occurrence of new cases of disease when compared with similar vineyards that were not so rogued. Also the annual incidence of Pierce's disease was not apparently influenced in two additional 10-acre plots where the diseased vines were removed several times each season over a period of five years.

Control of brown rot and oleocellosis of citrus fruit in the packinghouse. L. J. KLOTZ and G. A. ZENTMYER. If brown rot infection of lemons (incubation at 60°–65° F.) took place more than 10 hr. previous to immersion in cold fungicides the decay was unchecked. If the period of incubation (60° F.) was 30 hr. or less brown rot could be stopped by 4-min. immersion in water at 120° F. To endure the hot immersion without liberating rind oil and suffering the surface breakdown called oleocellosis, lemons must be slightly wilted by letting them stand 3 to 7 days after picking. Storing lemons at 40 per cent relative humidity, 70°–75° F., in air with rapid movement did not adequately protect them from rind oil spotting during subsequent immersion at 120° F. Exposing inoculated lemons to hot moist air (100°–104° F., 95 per cent R.H.) not only stopped brown rot even after 36-hr. incubation at 53° F. but conditioned the fruit so that no rind oil was liberated during the hot immersion. Lemon oil allowed to remain one or more seconds in contact with the surface of silver or green lemons and then removed with warm soda ash-soap solution caused definite injury.

Effects of curly-top virus strains on extent of injury in root tips of susceptible and resistant sugar beets. C. F. LACKEY. Three strains of curly-top virus were used. Strain 1, a virulent one, causes severe vein roughening and leaf distortion on susceptible beets; strain 2, a less virulent one, produces very little dwarfing and only mild vein roughening of susceptible beets; strain 6 produces very little vein roughening or leaf distortion but does greatly dwarf susceptible beets and causes death in a high percentage of cases. Susceptible beet root tips infected with strain 1 show marked degeneration and necrosis of the cells surrounding the sieve tubes. The resistant beet tips show only an occasional cell with some degeneration. Strain 2 produces only mild degeneration in a few of the cells surrounding sieve tubes and this degeneration is about equal in amount in root tips of susceptible and resistant beets. Strain 6 apparently damages susceptible beets by extending injury to their root tips rather than to their leaves, as is true of strain 1. The severe degeneration and necrosis it causes involves many other cells besides those surrounding the sieve tubes. The injury in the root tips of resistant beets is of the same type as in those of susceptible beets but less severe.

Comparative growth in culture of eleven physiologic races of Ustilago bullata. J. P. MEINERS. In an effort to determine the effect of various temperatures on the cultural

characteristics of head smut of grasses (*Ustilago bullata*), monosporidial lines of 11 physiologic races were grown for 4 weeks in cultural chambers at a uniform relative humidity of 81 per cent and at constant temperatures of 5, 10, 15, 20, 25, and 30° C. Each of the 11 races was represented in the study by 2 monosporidial lines of opposite sex derived from the same chlamydospore. In general, the results showed that not only does temperature influence rate of growth tremendously, but also that the individual races apparently vary in their cardinal temperatures. The optimum temperatures for cultural growth of most races were at 20° and 25° C. Some races grew well over a wide range of temperatures, whereas others appear to be adapted to a narrower range. Color and topography varied greatly with changes in temperature. Monosporidial lines from the same chlamydospore but of different sex reacted alike to changes in temperature.

Effect of daylight on volatile toxicity of lime-sulphur to fungi. JOHN I. MIRHIJ. Turbidity of 0.05 to 0.5 per cent of lime-sulphur increased with increasing light intensities. Hydrogen sulphide evolution was greater in dark than in light from 0.01 per cent lime-sulphur. Germination of conidia of barley mildew (*Erysiphe graminis*) was almost completely inhibited in 5 hours in vapors from 0.1 per cent lime-sulphur in daylight, but inhibition was less in darkness, and less with increasing or decreasing lime-sulphur concentrations, except for those of 0.001 to 0.01 per cent, where inhibition was greater in darkness; and for concentrations of 10 to 100 per cent lime-sulphur, where light had no effect on toxicity. For bean leaves infected with rust (*Uromyces phaseoli*) for 4–5 days before treatment, the same relations with respect to light and lime-sulphur solution applied. Hydrogen sulphide produced from ferrous sulphide and sulphuric acid was more toxic to bean rust and more injurious to bean leaves in light than in darkness, while chemically produced sulphur dioxide was more toxic to bean rust in darkness. Complete eradication of bean rust without injury was obtained from hydrogen sulphide but not from sulphur dioxide.

An indicator agar for the determination of the relative concentration of ascorbic acid in potato tuber tissue. WM. NEWTON. An indicator agar that reveals the approximate concentration of ascorbic acid in potato tuber tissue by direct contact is prepared by heating together 2 gm. agar, 75 ml. water, 10 ml. of a 10 per cent solution of potassium iodide, and 5 ml. of a 1 per cent solution of soluble starch. After cooling to 60° C., 5 ml. of glacial acetic acid and 5 ml. of an 0.01 N solution of potassium iodate are added and the mix is immediately poured into Petri dishes. When uniform filter-paper discs saturated with standard ascorbic acid solutions or uniform tuber-tissue discs are placed upon the agar surface, after 24 hr. at 5° C. the size of the area decolorized is directly related to the concentration of ascorbic acid in the solutions and tissue discs. Tissue discs from tubers affected with mosaic and leaf roll decolorize a greater area than discs from normal tubers from the same variety, and the decolorization is independent of tuber size. The diagnostic accuracy of the method is further strengtnened by the tendency of virus infected tissue to remain white and healthy tissue to become blue-black.

Fungi causing root rots of cereals in California. JOHN W. OSWALD. Isolations over a six-year period have shown that eight pathogenic fungi are involved in the cereal root rot complex in California. The disease has been found in 114 fields representing 16 counties. Widespread on wheat and barley in the order of their apparent importance are *Helminthosporium sativum*, *Fusarium graminearum* (*Gibberella saubinetii*), *Fusarium culmorum*, *Ophiobolus graminis*, and *Fusarium nivale* (*Calonectria graminicola*). *Pythium graminicolum* and *Sclerotium rolfsii* were found in local areas on barley and *Helminthosporium avenae* similarly on oats. The seedling blight and adult root rot phases of the five common pathogens are severe on wheat and barley, whereas oats have proven tolerant to all but *F. culmorum*. *H. sativum* and *F. graminearum* are frequently found together in the same field and often in the same host. Perithecia of *F. graminearum* have been observed in the field only once, their failure of development probably being due to low humidity when temperatures are sufficiently high. The absence of wheat or barley scab in the State is explained by lack of ascospore inoculum and inadequate humidity at heading time. *Wojnowicia graminis*, *Fusarium equiseti*, and *F. scirpi*, though commonly associated with the disease, have proven to be only of secondary importance.

Similarities in the pathological anatomy of Quick-Decline- and Tristeza-diseased orange trees. HENRY SCHNEIDER, A. A. BITANCOURT, and VICTORIA ROSSETTI. Radial sections of the phloem at the bud union in mature trees suffering from the two diseases have been compared. In the early stage of both diseases, the sieve tubes and companion cells below the union became necrotic and occasionally parenchyma cells became hypertrophied. In later stages the older sieve tubes above the union were also necrotic. Intensified cambial activity on the phloem side at the bud union resulted in a bulge on the

cambial face of the bark in advanced stages of the disease. Such hyperplastic tissue was similar to normal phloem; but the cells were smaller in size, and the sieve tubes became necrotic on reaching maturity or in some cases at a later date. New sieve tubes below the union less often became necrotic than those in the vicinity of the union, and no hyperplasia occurred there. Occasionally parenchyma cells above the union divided to produce a callus-like tissue. No differences in the anatomy of the two diseases were noted.

Rhizoctonia solani on field crops in the West. RODERICK SPRAGUE. Nearly 4 per cent of all fungi isolated from the roots of Gramineae in the northern part of the western United States were *Rhizoctonia solani*. Symptoms included seed, root, stem, and culm rots. Inoculations in the greenhouse at Mandan, North Dakota, and at Pullman, Washington, during 1940 to 1947 inclusive disclosed that five races of this species were recognizable by differences in their attacks on Kubanka wheat, Marion oats, blue grama, crested wheatgrass, Turghai proso millet, black amber sorghum, and Grimm alfalfa grown at 50°–70° F. Race 1 was virtually nonparasitic. Race 2 was weakly parasitic on alfalfa and proso but caused up to 90 per cent loss in blue grama and up to 16 per cent loss in oats and wheat. Race 3 was similar in reaction but could cause 50 per cent loss in sorghum and complete loss in alfalfa. Race 4, which was common on range grasses, especially bluegrasses, was highly pathogenic to all of the indicator plants, causing a seed and root rot. Race 5, present in the coast region of Oregon and Washington, was also parasitic on all these hosts except alfalfa, which was highly resistant; but this race caused a culm rot after emergence.

Variations in response of fungi to fungicidal chemicals. GEORGE A. ZENTMYER and L. J. KLOTZ. The fungicidal efficiency of 25 organic chemicals was tested *in vitro* against diverse types of fungi. Closely related compounds differed greatly in effectiveness; 8-hydroxyquinoline, a metal-precipitating chemical, was highly fungistatic, while its isomer, 2-hydroxyquinoline, which does not react with metals, was low in toxicity to fungi. The fungicidal action of p-nitrophenol and o-nitrophenol paralleled the action of these chemicals in inhibiting polyphenol oxidase in higher plants. The action of p-nitrophenol against fungi may be the result of a similar enzyme inhibition. Marked specificity was noted in the action of some organic chemicals, including diphenyl, benzoic acid, Dithane (disodium ethylene bisdithiocarbamate), catechol alpha-terpineol, ethylene dibromide, Dowfume N (mixture of dichloropropane, dichloropropene). Such cosmopolitan saprophytes or facultative pathogens as *Trichoderma lignorum* and *Botryosphaeria ribis* (*Dothiorella gregaria*) were generally highly resistant to most chemicals tested. These fungi are evidently able either to tolerate or metabolize a wide variety of chemicals, as would be expected from their growth on diverse substrata. The age of culture used in mycelial transfers markedly affected the response of several fungi to fungicidal chemicals; transfers from young cultures (1 day old) were more resistant to fungicides than transfers from older cultures. Variations in effectiveness of fungicides on different culture media were also noted.

REPORT AND ABSTRACTS OF THE FIRST ANNUAL MEETING OF THE NORTHEASTERN DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The Northeastern Division held a very successful initial meeting at Amherst, Massachusetts, on November 26 and 27, 1946. This meeting was held in conjunction with the New England-New York Spray Specialists' Conference. Plant pathologists from all the Northeastern States and from different fields of interest comprised the 75 in attendance. Consequently there was active participation in the discussion meetings on Apple Scab, New Fungicides, Potato Diseases, and Dutch Elm Disease. Twelve formal papers were presented at one afternoon session. At the short business meeting the following officers were elected for 1947: President, S. E. A. McCallan; Vice-President, M. T. Hilborn; Secretary-Treasurer, W. T. Schroeder; Councilor, Thomas Sproston, Jr.

ABSTRACTS OF PAPERS PRESENTED AT THE MEETING

Nematodes on Tobacco in Connecticut. ANDERSON, P. J. Nematodes, serious pests of tobacco in the South, have not previously been reported on tobacco in Connecticut. In the summer of 1946 a severe infestation of the root-knot nematode (*Heterodera marioni*) caused severe stunting of shade tobacco in one field in Windsor. On other fields two other species of nematodes were found associated with deterioration of the roots and stunted growth of the plants. One of these was identified as the meadow nematode (*Pratylenchus pratensis*). How widespread nematode infestation may be in the tobacco fields of New England is not known because (1) a survey has not been made, and (2) nematode damage may have been attributed to other disease organisms.

Control of Tobacco Mildew. ANDERSON, P. J. Dusting tobacco seed beds with a 20–80 Fermate (ferric dimethyl dithiocarbamate) dust gave as complete control of mildew as did the standard Fermate spray, 1–50. Two bismuth subsalicylate spray preparations gave equally good control. The protective action of the bismuth preparations did not last longer than that of Fermate.

The Interference Phenomenon Between the Rib-Grass and Tobacco-Mosaic Viruses in Bean. BEALE, HELEN PURDY. When the rib-grass virus (*Marmor tabaci* H. var. *plantaginis* H.) is rubbed on the leaves of Early Golden Cluster bean (*Phaseolus vulgaris* L.), no systemic infection results and no local necrotic lesions occur, such symptoms that are characteristic of tobacco-mosaic virus (*Marmor tabaci* H.) on this host. In spite of the noninfectiousness of the rib-grass virus to Early Golden Cluster bean, whenever this virus is mixed in increasing concentration with relatively smaller quantities of tobacco-mosaic virus, the number of local lesions capable of resulting from the inoculation of tobacco virus alone is successively reduced. Because of this interference phenomenon existing between the two viruses, a preparation of rib-grass may contain a trace of tobacco-mosaic virus which is nondetectable by inoculation of bean, the usual criterion for freedom from contamination with tobacco virus. If the sample of rib-grass virus to be tested is inoculated first in tobacco (*Nicotiana tabacum* L.), in which both viruses multiply systemically, a sufficiently high concentration of tobacco virus will be produced to permit detection upon subsequent inoculation of bean although only a trace may be present in the original sample.

Performance of New Fungicides for Control of Potato Late Blight. DAVIDSON, R. S. and A. E. RICH. Comparative studies on the performance of some new organic fungicides for the control of potato late blight have been conducted at the Rhode Island Agricultural Experiment Station during 1945 and 1946. In 1945, 12 different synthetic organic materials were compared with Bordeaux. None of the sprays effected as complete control as did Bordeaux. Phygon (2,3-dichloro 1,4-napthoquinone), Fermate (ferric dimethyl dithiocarbamate), Dithane 14 (disodium ethylene bisdithiocarbamate), and Zerlate (zinc dimethyl dithiocarbamate) were significantly better than the check treatments. Fungicidal properties of 7 organic materials were further investigated in 1946. Again, Bordeaux proved superior to all other chemicals when applied as foliage sprays. However, Zerlate, Phygon, and G–11 (di-trichloro-dihydroxy diphenyl methane) were significantly better than

the check treatments. It was observed in both seasons that materials which effected significant control over the checks produced less injury to the foliage than did the copper containing sprays. The failure of these organic sprays to control late blight apparently was due in part to their poor adherence. In 1945 and 1946 DDT was applied in all treatments, and in 1946 a control fungicide plot was included which received only DDT. By comparison of a control which received neither a fungicide nor an insecticide with the control which received only DDT, it was evident that DDT did not possess fungicidal properties, while G–11, which is similar chemically to DDT, proved to have fair fungicidal properties.

Oxyquinoline Benzoate Aids Suppression of Symptoms of Dutch Elm Disease. DIMOND, ALBERT E. Late in July, 200 elms up to 6-inch diameter were inoculated basally with giant spore loads of *Graphium ulmi*. At this time half the trees were watered with 1: 1000 oxyquinoline benzoate and half with water. On August 20, foliage symptoms in the crown averaged 47.1 per cent for checks and 29.4 per cent for treated trees, a highly significant difference. During September, check trees dropped leaves earlier than treated ones. Three per cent of treated and 5 per cent of check trees failed to develop symptoms. Cultures were recovered from all but 14 per cent of treated and 5 per cent of check trees. Some of these showed no symptoms. Effects were not of practical magnitude in this experiment, but the method of inoculation was much more severe than in nature. Wilted diseased trees to which oxyquinoline benzoate was applied recovered from wilt, and appearance of further symptoms was arrested. Apparently fewer trees succumb to the disease if treated and more trees recover when treated with oxyquinoline benzoate. As shown by culture recovery, this treatment does not kill the fungus in the diseased tree.

Fungicides Applied in Fertilizer for the Control of Cabbage Clubroot and Damping-off. DORAN, W. L. Fungicides were mixed with a 5: 8: 7 commercial fertilizer and this was applied to soil at the rate of 15.6 gm. of the fertilizer per square foot immediately before seeding. The method is convenient, especially in the case of an application too light to be distributed evenly unless in some carrier. Dithane D–14 (disodium ethylene bisdithiocarbamate) and Dow Seed Protectant No. 9 (zinc trichlorophenate) thus applied controlled damping-off better or with less injury than when applied in water immediately after seeding. Mercurous chloride and other mercury salts (0.15 or 0.20 gm. per square foot) controlled clubroot better and more safely in soils which had previously received an application of hydrated lime (20.0 gm.) or sodium chloride (10.0 gm. per square foot) than they did in untreated soil. And Dithane was more effective against damping-off in a limed soil. But the fungicides named above, as well as Fermate (ferric dimethyl dithiocarbamate), Phygon (2,3-dichloro 1,4-naphthoquinone), and zinc mercaptobenzothiazole, were all less effective against clubroot than was Tuads (tetramethyl thiuram disulfide). Tuads, 0.55 gm. per square foot, applied in fertilizer, gave good control of clubroot and of damping-off and markedly improved the growth of cabbage seedlings in both limed and unlimed soils.

Eradicating Apple Foliage Scab With Summer Sprays. GUBA, E. F. The action of summer eradicant sprays on a block of 19-year-old McIntosh trees has been studied. Following applications, mass spore germination tests were made at frequent intervals to determine the action of the sprays. The trees were sprayed at 450-lb. pressure, and 15 to 16 gallons were applied per tree. Liquid lime-sulfur (1–50) gave fair results. Two to three applications almost completely eradicate scab. The treatments are accompanied by more or less injury to the fruit and foliage. Puratized Agricultural Spray (phenylmercuri triethanol ammonium chloride) ($\frac{1}{2}$ pint–50 gal. water) operates to kill the incubating and sporulating scab fungus and to stop infection completely without any apparent injury to the foliage. Both fungicides cause the collapse of the conidia and change the color of their contents. The conidia are inactivated completely following applications of Fermate (ferric dimethyl dithiocarbamate) ($\frac{3}{4}$ lb.–50 gal. water) without accompanying changes in structure or color of the spores. Negligible spore germination was maintained throughout the season by 6 applications beginning at calyx on May 17 in one set of trees, and 3 applications beginning on June 10 in another set of trees. The character of the scab mold on the foliage appears to remain healthy, suggesting fungistatic rather than fungitoxic action. The action of Phygon (2,3-dichloro 1,4-napthoquinone) ($\frac{1}{2}$ lb.–50 gal. water) was somewhat comparable with that of Fermate although inactivation was not so complete. Wettable sulfurs lack scab eradicant action. None of the materials eradicated fruit scab lesions.

The Characteristic Curve for the Action of Copper Sulphate on the Germination of Spores of Sclerotinia fructicola and Alternaria oleracea. MCCALLAN, S. E. A. Extensive data were obtained for the dosage response curve of copper sulphate on spore germination inhibition of *Sclerotinia fructicola* and *Alternaria oleracea*. Thirty-four doses, from 0.105 to 32.000 p.p.m. Cu, with a dose ratio of $\sqrt[4]{2}$ were used, 1000 spores counted at each dose and the entire test run 3 times, giving about 100,000 spores for determining the characteristic curve. Elaborate precautions are necessary for defining germination and ascertaining prior germination and nongermination of control spores. The final percentage

inhibition of germination for treated spores is derived from the formula $100 - \frac{100\ (T-P)}{C-P}$ where C and T are percentage observed germination in controls and in treated spores, respectively, and P is percentage of prior germination in control. Smooth curves concave upward are obtained on logarithmic probability paper. By means of Parker-Rhodes α transformation straight lines result for the regression of probits on conc. α. For *S. fructicola* $\alpha = 0.41$ and $E = 1.354\ X + 0.590$, for *A. oleraceae* $\alpha = 0.56$ and $E = 0.525\ X + 1.873$. However, a negative α transformation is required to straighten convex curves. The Langmuir adsorption equation when applied above gives concave curves. Germ tube length expressed as percentage of control plotted against percentage germination gives a sigmoid curve.

1946 Potato Spray and Dust Experiments in New York State. NIEDERHAUSER, J. S., W. A. RAWLINS, and A. M. FRENCH. Seven large-scale experiments comparing potato fungicides in combination with standard amounts of DDT were conducted in the important potato-growing areas of New York State. Dithane (D–14) (disodium ethylene bisdithiocarbamate) ranked first in three of four spray experiments, and Dithane (HE–178) was first in two of three dust experiments. The yields obtained with fixed coppers were about equal to those obtained with Bordeaux mixture spray or copper lime dust. Zerlate (zinc dimethyl dithiocarbamate) failed to control late blight in the one test where it was included. However, late blight was not an important factor affecting yield in any of these experiments, and appeared late in the season if at all. In a replicated experiment covering 30 acres of Chippewa potatoes, airplane dusting did as well as ground dusting in late blight and insect control. Lower yields from ground-dusted fields were attributed directly to wheel-row damage. Detailed studies on distribution of the dust applied at 40 lb. an acre and in a 30-foot airplane swath showed 4.7 milligrams of copper deposited per square foot directly under the plane, and 2.9 milligrams of copper deposited per square foot at a distance of 15 feet from the center of the swath.

Some Problems Involved in Use of 2,4-D as a Tomato Defoliant. SCHROEDER, W. T., and F. G. SMITH. Further field trials confirmed the previous year's results that 2,4-D reduced the cracking of ripe fruit occurring in periods of excessive precipitation. The amount of cracking was inversely proportional to the extent of defoliation or vine damage caused by 2,4-D and dependent upon its time of application. Results also showed that 2,4-D treatments may affect quality and yield of the product in other ways. Processed juice from treated fruit was inferior in flavor and color. The percentage of anthracnose was increased, especially in the absence of an adequate protective fungicide schedule. From the standpoint of avoiding frost damage, even the earliest applications of 2,4-D did not significantly increase the rate of ripening as determined by yield. In fact, these applications caused lower yields, probably by reducing fruit size. However, in excessively wet seasons, control of cracking and resulting fruit mold might offset this reduction in yield. These results emphasized the complexity of problems involved in 2,4-D treatment of tomatoes and suggest that other defoliants be similarly investigated.

PHYTOPATHOLOGICAL NOTES

A Chromatographic Method for the Detection of Tobacco-Mosaic Virus in Juice from Diseased Turkish Tobacco Plants.—A simple paper-chromatographic method has been developed for the detection of tobacco-mosaic virus in extracted juice of Turkish tobacco plants. A chromatographic method using paper has been described by Consden, Gordon, and Martin[1] for the separation of mixtures of amino acids. It has been modified somewhat in testing for tobacco-mosaic virus.

The apparatus used is shown in figure 1. A glass cylinder (1) is used as a support for a large rubber or cork stopper (2) on which is placed a porcelain embedding dish (3). Glass microscope slides (4) are inserted in cuts made in the stopper on each side of the dish to support the paper strips (5). Whatman no. 1 filter paper, 2×8 inches, is used in the test. This apparatus is placed in a large glass jar fitted with a tight cover. A small amount of water is placed in the bottom of the jar to give a saturated atmosphere for the tests.

Several color reactions were tried experimentally with purified tobacco-mosaic virus. The Sakaguchi[2] arginine reaction appeared to be the most sensitive color test for the virus on Whatman no. 1 filter paper. The reagents for the test are used in the following order: *1*) 10 per cent aqueous potassium hydroxide; *2*) 0.1 per cent alpha naphthol in 50 per cent ethyl alcohol; *3*) 5.25 per cent sodium hypochlorite (undiluted Chlorox). When these reagents are applied to the filter paper by means of an atomizer, presence of arginine is indicated by the appearance of a bright pink color in about one minute. The color remains for several minutes and then fades.

Since arginine is also present in normal plant proteins, the success of the test depends upon the ability of the solvent system to move the virus to a given spot on the paper strip while not moving normal arginine-containing proteins to that same spot. To make the test a drop of plant juice is placed $1\frac{1}{2}$ inches below the top of the paper strip. Juice from plants known to be healthy may be placed on the same paper strip and used as a control. The paper is folded at a point 1 inch below the top. It is hung on the edge of the glass slide with the shorter portion extending into the solvent dish and the longer portion hanging freely in the atmosphere of the jar. When the solvent is added to the dish it is taken up rapidly by the paper. If the proper solvent is used the virus will be moved to a new location by the liquid as it passes down the paper. When the advancing front of the solvent approaches the bottom of the paper, the paper strip is removed and dried. The paper is then treated with the arginine reagents to indicate the position of the virus.

[1] Consden, R., A. H. Gordon, and A. J. P. Martin. Qualitative analysis of proteins. A partition chromatographic method using paper. Biochem. Jour. 38: 224–232. 1944.

[2] Sakaguchi, S. A new color reaction of protein and arginine. Jour. Biochem. (Japan) 5: 25–31. 1925.

It was found that water buffered at a pH level of 4 or lower would not move the virus while water buffered at a pH level of 4.5 or higher moved tobacco-mosaic virus to give a satisfactory color test. When buffers having a pH level of 6 to 7 were used as solvents, very satisfactory color tests were obtained and the transported virus was recovered in a highly active form. The best recovery procedure was to run two strips simultaneously under the

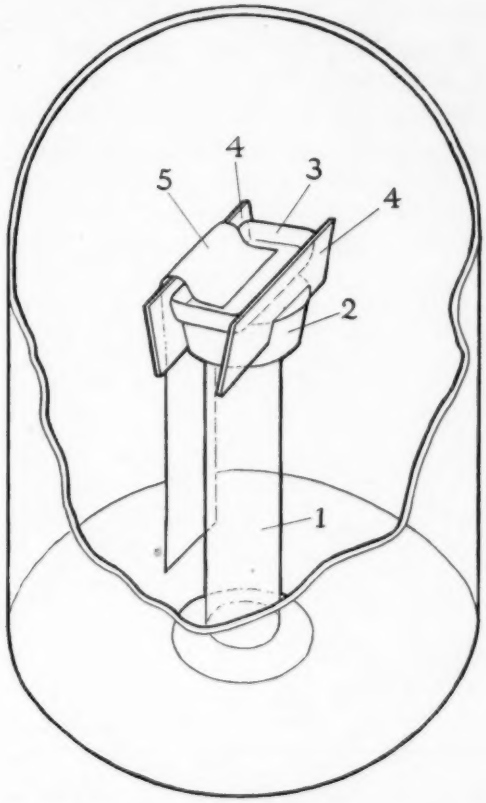

FIG. 1. Cut-away view of large glass jar showing the apparatus used for the test. A glass cylinder (1) supports a large rubber or cork stopper (2), on which is placed a porcelain embedding dish (3). Glass microscope slides (4), inserted in cuts made in the stopper, support the paper testing strip (5). (Drawing by J. A. Carlile.)

same conditions. The position of the virus was located by applying the color reagents to one of the strips. The virus location was then outlined with pencil on the other strip. The spot was cut out and the virus was removed from the paper by elution with buffer. When water buffered at a pH level of 4.5 or higher was used as the solvent system, most of the normal arginine-containing proteins and the chlorophyll remained at the original site of application and did not interfere with the color test for the virus.—G. W. COCHRAN, Department of Animal and Plant Pathology, The Rockefeller Institute for Medical Research, Princeton, New Jersey.

The Fungicidal Value of Mixtures of Lime Sulphur and Zinc Sulphate.—The discovery by Heuberger[1] that the addition of zinc sulphate increases the fungicidal value of disodium ethylene bisdithiocarbamate suggested that a similar effect might result if zinc sulphate is added to lime sulphur. Such a mixture is already in successful use as a combined insecticide and zinc deficiency spray[2] and it is apparently a more efficient insecticide than the same mixture without zinc.

A series of concentrations of zinc sulphate was added to a series of concentrations of lime sulphur and the mixtures tested as eradicant and protective sprays on a number of plants and diseases by methods previously described.[3] Data were plotted as lime-sulphur dosage against percentage

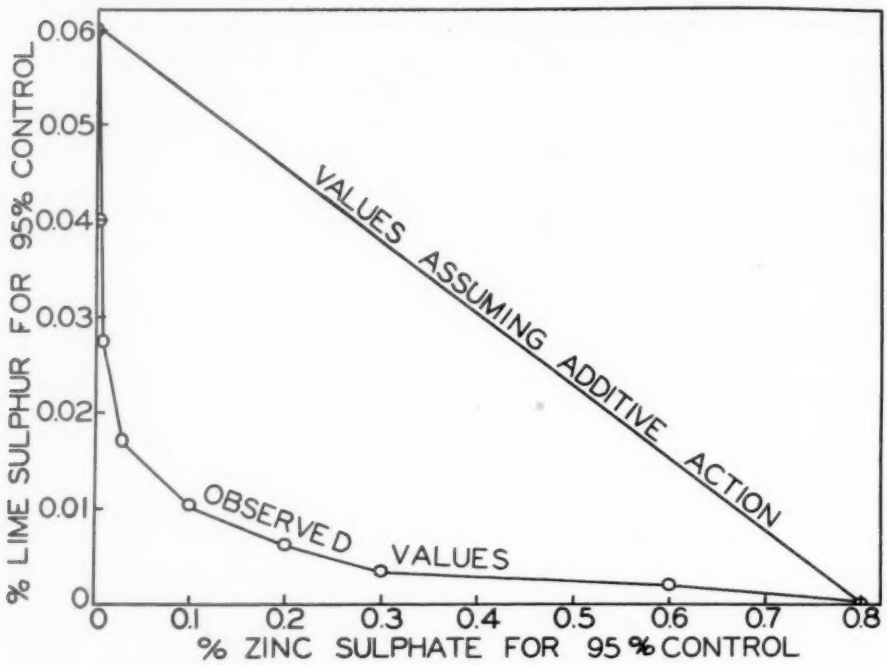

FIG. 1. The protective value for bean rust of mixtures of lime sulphur and zinc sulphate.

of disease control, and the dosage of lime sulphur for 95 per cent control with each concentration of zinc sulphate was determined. The greatest synergism observed was in greenhouse tests with bean rust, and average results of 15 trials are summarized in figure 1. In these tests the LD 95 for lime sulphur alone was 0.06 per cent concentration of applied spray. The addition of zinc sulphate decreased the LD 95 at all concentrations

[1] Heuberger, J. W. Factorial studies on Dithane plus zinc sulphate-lime: "the reaction product" zinc ethylene bisdithiocarbamate. Phytopath 36: 685–686. 1946.

[2] Lewis, H. C. Spray injury from zinc–lime sulphur in Central California. Calif. Citrograph 31: 112. 1946.

[3] Yarwood, C. E. The function of lime and host leaves in the action of Bordeaux mixture. Phytopath 33: 1146–1156. 1943.

tested. With 0.1 per cent zinc sulphate the LD 95 was 0.01 per cent lime sulphur, or about one sixth that required when lime sulphur was used alone or one fifth that expected of the combination if additive action is assumed. Other uses in which mixtures of zinc sulphate and lime sulphur have been more fungicidal than expected on the basis of additive action were as eradicants for bean rust, as protectants against bean powdery mildew, as protectants against hop downy mildew, and as protectants against snapdragon rust, though in all these cases the synergistic effect was less than in the case of bean rust.

Iron sulphate and copper sulphate were also tested separately as supplements to lime sulphur and disodium ethylene bisdithiocarbamate. All combinations were synergistic for bean rust but none so greatly synergistic as zinc sulphate with these same fungicides. As protectants and eradicants for bean and cucumber powdery mildews, however, the iron sulphate-lime sulphur combination may have been as effective as the zinc sulphate-lime sulphur mixture.—C. E. YARWOOD, University of California, Berkeley, California.